

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
17 May 2001 (17.05.2001)

PCT

(10) International Publication Number
WO 01/34186 A1

(51) International Patent Classification: **A61K 39/00**,
C07H 21/04, C12P 21/06, C12N 15/00, G01N 33/00,
C07K 16/00

(74) Agents: **CORLESS, Peter, F. et al.**; Dike, Bronstein,
Roberts & Cushman, Edwards & Angell LLP, Intellectual
Property Group, 130 Water Street, Boston, MA 02109
(US).

(21) International Application Number: **PCT/US00/30968**

(22) International Filing Date:
12 November 2000 (12.11.2000)

(81) Designated States (national): **AU, CA, JP**.

(25) Filing Language: **English**

(84) Designated States (regional): **European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR)**.

(26) Publication Language: **English**

(30) Priority Data:
60/165,226 12 November 1999 (12.11.1999) **US**

Published:

- *With international search report.*
- *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

(71) Applicant and

(72) Inventor: **LEHRER, Samuel, B.** [US/US]; 400 Sharon Drive, New Orleans, LA 70118 (US).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(72) Inventor: **REESE, Gerald**; 3520 Camphor Street, New Orleans, LA 70118 (US).



WO 01/34186 A1

(54) Title: **COMPOSITIONS AND METHODS FOR MODIFYING AN IMMUNE RESPONSE AGAINST TROPOMYOSIN**

(57) Abstract: Disclosed are compositions and methods for modifying an immune response against tropomyosin and related antigens. In one embodiment, the invention provides vaccines including modified tropomyosin that reduce or eliminate an unwanted immune response against tropomyosin. Also provided are modified tropomyosin molecules with modified antibody binding sites (epitopes) that significantly reduce or eliminate potential to engage the immune system. The invention also provides transgenic animals, particularly transgenic shrimp, that include at least one of the modified tropomyosin molecules described herein. The invention has a wide spectrum of uses including reducing harmful immune responses to crustacea, arthropods, and other animals.

5

**COMPOSITIONS AND METHODS FOR MODIFYING AN IMMUNE
RESPONSE AGAINST TROPOMYOSIN**

10

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to compositions and methods for modifying an immune response against tropomyosin and related antigens. In one embodiment, the invention provides vaccines including modified tropomyosin that reduce or eliminate an unwanted immune response against tropomyosin. Also provided are modified tropomyosin molecules with modified antibody binding sites (epitopes) that significantly reduce or eliminate potential to engage the immune system. The invention also provides transgenic animals, particularly crustacea, that include at least one of the modified tropomyosin molecules described herein. The invention has wide applicability including reducing harmful immune responses to crustacea, arthropods, and other animals.

2. Background

Allergic disease is a common health problem affecting humans and companion animals (mainly dogs and cats) alike. Allergies exist to foods, molds, grasses, trees, insects, pets, fleas and other substances present in the environment. Some allergic reactions, especially to foods and insects can be so severe so as to be life threatening.

30

There is almost universal recognition that most allergies are manifested by the release of histamines and other mediators of inflammation by mast cells and basophils which are triggered into action when IgE antibodies bound to their receptors on the mast cell surface are cross-linked by allergan. Other than avoidance, and drugs, for example, antihistamines, decongestants, and steroids, that only treat symptoms and can have unfortunate side effects and often provide only, temporary relief, the only currently medically accepted treatment for allergies is immunotherapy. Immunotherapy involves the repeated injection of allergan extracts over a period of years to desensitize a patient to the allergan.

Unfortunately, traditional immunotherapy is time consuming, usually involving

- 5 years of treatment, and often fails to achieve its goal of desensitizing the patient to the allergan.

Among allergans of animal origin, shellfish (crustaceans and mollusks) are a frequent cause of allergic reactions to foods. Most shellfish species that elicit
10 allergic food reactions belong to the class crustacea and include shrimp, crab, crawfish and lobster; the shrimp genera *Penaeus* and *Metapenaeus* have been two of the most frequently studied. The only major allergan reported in shrimp is the muscle protein tropomyosin. At least 80% shrimp-allergic subjects react to tropomyosin and it binds approximately 85% of the shrimp-specific IgE from
15 shrimp-allergic subjects; all other shrimp allergans bind IgE from less than 25% of the shrimp-allergic subjects. Recent studies have demonstrated that tropomyosin is an important allergan in other crustaceans such as lobster *Panulirus stimpsoni* and *Homarus americanus* (Pan s 1, Hom a 1), crab *Charyabdis feriatus* (Cha f 1), mollusk such as squid *Todareus pacificus* (Tod p 1), snail *Turbo cornutus* (Tur c 1)
20 and oyster *Crassostrea gigas* (Cra g 1) and in other invertebrates such as house dust mite *Dermatophagoides farinae* (Der f 10) and *D. pteronyssinus* (Der p 10), and cockroach *Periplaneta americana* (Per a 7). Furthermore, concomitant clinical and *in vitro* hypersensitivity to crustaceans, insects, arachnids, mollusks and even nematodes have implied tropomyosins as the cause of clinical cross-sensitivity
25 among invertebrates.

It has been reported that tropomyosin belongs to a family of proteins present in all eukaryotic cells, where it is associated with the thin filament in muscle, and microfilaments in many non-muscle cells. Together with actin and myosin,
30 tropomyosin plays a role in the contractile activities of these cells, as well as in the regulation of cell morphology and motility. Tropomyosin is thought to be present in phylogenetically unrelated vertebrate and invertebrate species, with several tropomyosin isoforms being found in muscle (skeletal, cardiac and smooth), and non-muscle cells such as those in brain, fibroblasts and platelets. Even though the
35 degree of sequence identity and functional similarity is reported to be very high among tropomyosins, vertebrate tropomyosins are generally considered to be non-allergenic.

Tropomyosins have attracted substantial research interest.
40

- 5 For example, there is understanding that the proteins are coiled-coil dimers made up of two parallel α -helical tropomyosin molecules that are wound around each other. The tropomyosin monomer contains a heptad repeat, (abcdefg) in which generally large hydrophobic non-polar residues occur at positions a and d, while positions b, c, e, f and g are usually occupied by polar or ionic amino acids.
- 10 The interaction between two alpha-helices in a coiled-coil involve these hydrophobic residues in position a and d. Also charge-charge interactions between acidic residues found in position e and basic residues in position g help also stabilize the coiled-coil. Outer positions b, c, f, must be free to interact with proteins such as actin and troponin. Interestingly, the tandem repeats always occupy position d, e, f,
- 15 g, and a in the heptameric repeats, which represents the area of interaction between the two α -helices of tropomyosin. Although tropomyosin is a very flexible molecule that may spontaneously unfold and expose those internal sequences, the repeats are thought to be mostly non-exposed sequences in the native molecule.
- 20 Tests such as skin prick test (SPT) have been used to gauge risk of allergy to tropomyosin and other molecules.

There remains a need for a safe and efficacious therapy for allergies, especially where traditional immunotherapy is ill advised due to risk of anaphylaxis to the patient or lack of efficacy. There is also a need for alternatives to therapies, for creating foods, materials or substances which contain modified allergens that do not elicit a harmful allergic response.

SUMMARY OF THE INVENTION

- 30 The invention generally relates to compositions and methods for modifying an immune response against tropomyosin. As discussed below, the invention has many important uses including reducing or eliminating harmful immune responses against the tropomyosin of animals such as crustacea and arthropods. Also provided are transgenic animals and especially transgenic crustacea that include at
- 35 least one recombinant tropomyosin specifically modified to reduce or eliminate the unwanted immune responses.

In particular, we have discovered tropomyosin sites (epitopes) that engage the vertebrate immune system and elicit harmful immune responses. More

40 specifically, we have identified the Pen a 1 (tropomyosin) epitopes that elicit IgE-

5 mediated immune responses. As related below, we have found about 15 - 20 IgE binding regions in the tropomyosin molecule. We have found one epitope for region 1, one epitope for region 2, two epitopes for region 3, one epitope for region 4 and three epitopes for region 5.

10 It is thus an object of the invention to provide tropomyosin molecules in which at least one of the IgE binding regions has been modified to reduce or eliminate IgE antibody binding.

Accordingly, and in one aspect, the invention provides vaccines that include at least one modified tropomyosin that reduces or eliminates an unwanted immune
15 response against tropomyosin. Such a modified tropomyosin can, in one embodiment, include at least one amino acid substitution in at least one of the foregoing regions one to five of tropomyosin. Other vaccines according to the invention will include at least a fragment of tropomyosin and may also include at least one of the modified tropomyosins described herein.

20 In a particular embodiment, the vaccine will include at least one modified amino acid that reduces binding, preferably specific, between crustacea tropomyosin and an IgE antibody of interest by at least about 45% as determined by a standard IgE antibody test. Preferably, suitably modified amino acid
25 sequences will include at least one epitope with at least one amino acid substitution that reduces the binding according to the test. Examples of such preferred amino acid substitutions include those which remove at least one of a non-polar aliphatic, polar uncharged, aromatic, positively charged or negatively charged group from the amino acid.

30 As discussed, another preferred vaccine includes crustacea tropomyosin or an IgE antibody eliciting fragment thereof. In this invention embodiment, exposure to the tropomyosin is thought to condition the immune system against significantly engaging the molecule as part of a harmful response.

35 Also provided are modified tropomyosin molecules such as peptides with modified antibody binding sites (epitopes) that significantly reduce or eliminate potential to engage the immune system. The invention also provides transgenic animals, particularly crustacea, that include at least one of the modified
40 tropomyosin molecules described herein. The invention has wide applicability

- 5 including reducing harmful immune responses to crustacea, arthropods, and other animals.

Tropomyosin is an essential muscle protein that is present in all animal species and is highly conserved. Thus, invertebrate tropomyosins have amino acid
10 sequence homology with vertebrate tropomyosins such as those present in non-allergenic foods such as beef, pork and chicken. We have used the homologous region of non-allergenic tropomyosin as a template to alter the allergenic epitopes of shrimp tropomyosin to render them inactive. The aim was to make minimal changes in the allergenic epitopes that would induce maximal reduction of
15 tropomyosin-specific IgE binding.

In one embodiment, the invention provides a series of 46 overlapping peptides that span the entire 284 amino acid residue of Pen a 1 tropomyosin protein. Each of these peptides were used to identify the IgE binding epitopes of
20 Pen a 1. As used herein, IgE binding epitopes are defined as any sequence of Pen a 1 that binds Pen a 1-specific IgE of shrimp allergic subjects.

In another embodiment, the invention provides for peptides that have reduced or totally lack binding ability to Pen a 1-specific IgE. Peptides were
25 generated that had one or more amino acid substitutions based on, but not limited to, sequence comparisons with non-allergenic tropomyosins. As used herein, modified Pen a 1 peptides or Pen a 1 molecules with reduced or abolished IgE binding capacity are defined as any Pen a 1 molecule or peptide that contain one or more amino acid substitutions that reduce or abolish IgE binding of the peptide or
30 Pen a 1 molecule.

In other preferred embodiments, the present invention provides for oral and/or immunotherapy using the modified Pen a 1 peptides or molecules, for example, a vaccine expressing the modified peptides or Pen a 1 molecules. In
35 addition, modified tropomyosin used for the development of transgenic shrimp, crab, lobster, or crawfish in which the native tropomyosin production is reduced allowing for production of hypoallergenic seafood that will have reduced or abolished potential to induce allergic reactions.

40 Other aspects of the invention are disclosed *infra*.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the forty six, synthetic overlapping peptides spanning the entire sequence of Pen a 1 (length 15 amino acid residues, offset: 6 amino acids).

10

Figure 2 shows the results of the PepScan analysis of Pen a 1 peptides sera from 18 shrimp allergic subjects. (Peptide length: 15 amino acids, offset: 6 amino acids).

15

Figure 3 (3.1 - 3.5) shows the individually recognized epitopes and sequence comparison with allergenic and non-allergenic tropomyosins in the Pen a 1 regions 1-5.

20

Figure 4 shows the peptide amino acid sequence resulting from combinatorial substitutions and transforming a Pen a 1 peptide into the homologous chicken tropomyosin (TM) sequence.

Figure 5 is an autoradiograph showing the reactivity of the combinatorial substituted peptides with IgE from individual sera..

25

Figure 6 illustrates the amino acid positions that are critical for binding to IgE.

Figure 7 shows the peptides recognized by the serum IgE of six shrimp allergic subjects, each spot representing a different peptide of all 46 tested.

30

Figure 8 shows the amino acid sequence comparison of Pen a 1 with other allergenic tropomyosins.

35

Figure 9 shows the sequence comparison of IgE-binding, recombinant peptides and non-IgE-binding synthetic peptides: Identical sequences are shaded.

Figure 10 shows 12 Pen a 1 varieties that contain substitutions in 78 positions and will reduce or abolish the IgE antibody reactivity of the Pen a 1 molecule.

40

DETAILED DESCRIPTION OF THE INVENTION

5 As discussed, the invention provides highly useful compositions and methods for modifying and particularly reducing a harmful or potentially harmful immune response against tropomyosin. Of interest are crustacea, arthropod, mollusk and arachnid tropomyosin, especially those of shrimp or insect origin.

10 The following definitions are used herein unless specified otherwise.

 An antigen is a molecule that elicits production of an antibody such as an IgE antibody.

15 An antibody or other similar term refers to whole immunoglobulin as well as immunologically active fragments which bind antigen. The immunoglobulins and immunologically active fragments thereof include an antibody binding site. Exemplary antibody fragments include for example, Fab, F(v), Fab', F(ab') fragments, "half molecules" derived by reducing the disulfide bonds of
20 immunoglobulins, single chain immunoglobulins, or other suitable antigen binding fragments (see, e.g. Bird et al., *Science*, pp. 242-424 (1988); Huston et al., *PNAS (USA)*, 85:5879 (1988); Webber et al., *Mol. Immunol.*, 32:249 (1995)). The antibody or immunologically active fragment thereof, may be of animal (e.g. a rodent such as a mouse or rat), or chimeric form (see Morrison et al., *PNAS (USA)*, 81:6851 (1984);
25 Jones et al., *Nature.*, 321:522 (1986)). Single chain antibodies of the invention can be preferred.

 An allergan is a subset of antigens which elicits IgE production in addition to other isotypes of antibodies.

30 An allergic reaction is one that is IgE mediated with clinical symptoms primarily involving the cutaneous (urticaria, angiodema, pruritus), respiratory (wheezing, coughing, laryngeal edema, rhinorrhea, watery/itching eyes), gastrointestinal (vomiting, abdominal pain, diarrhea), and cardiovascular (if a
35 systemic reaction occurs) systems.

 An epitope is a binding site including an amino acid motif of between approximately five to fifteen amino acids which can be bound by an immunoglobulin. A linear epitope is one where the amino acids are recognized in
40 the context of a simple linear sequence. A conformational epitope is one where the

- 5 amino acids are recognized in the context of a particular three dimensional structure.

An immunodominant epitope is one which is bound by antibody in a large percentage of the sensitized population or where the titer of the antibody is high,
10 relative to the percentage or titer of antibody reaction to other epitopes present in the same protein.

A decreased allergic reaction is characterized by a decrease in clinical symptoms associated with exposure to an allergen, which can involve respiratory,
15 gastrointestinal, skin, eyes, ears and mucosal surfaces in general.

The first step in making the modified allergan is to identify IgE epitope binding sites and/or immunodominant IgE binding sites. The second step is to mutate one or more of the IgE binding sites, preferably including at a minimum one
20 of the immunodominant sites. The third step is to make sufficient amounts of allergan for administration to persons or animals in need of tolerance to the allergan, where the modified allergan is administered in a dosage and for a time to induce tolerance, or for diagnostic purposes. The modified allergan can be administered by injection, or in some cases, by ingestion or inhalation.

25 Allergans typically have both IgE and IgG binding sites and are recognized by T cells. The binding sites of the allergans can be identified using phage display libraries to identify conformational epitopes (Eichler and Houghten, (1995) *Molecular Medicine Today*, 1:174-180; Jensen-Jarolim et al., (1997) *J. Appl. Clin. Immunol.* 101:5153a) or by using defined peptides derived from the known amino acid sequence of the allergan (see examples below).

It is desirable to modify the IgE-binding epitopes of the allergan. In order to reduce or abolish the IgE reactivity, the individual peptides containing IgE binding
35 epitopes are mutated into the homologous sequences of the allergenic (invertebrate) to the vertebrate (non-allergenic) tropomyosins. The substitutions and their effect on the IgE-binding capability of modified Pen a 1 peptides can be categorized according to different criteria. First, the minimal number of substitutions that a peptide must carry to render it non-reactive and second, the maximal number of
40 substitutions per peptide to allow the peptide to retain at least some IgE reactivity.

5

A modified allergan will typically be made using recombinant techniques. Expression in a prokaryotic or eukaryotic host including bacteria, yeast, and baculovirus systems are typically used to produce large (mg) quantities of the modified allergan.

10

A modified amino acid sequence, as used herein, refers to an amino acid sequence of tropomyosin which has been modified by substituting an amino acid such that modified amino acid sequence binding to an IgE molecule is reduced.

15

Substituted amino acids can be selected from a non-polar aliphatic group; a polar, uncharged group; an aromatic group; a positively charged group; or a negatively charged group. For representative amino acids from each group and a description of each group see Alberts et al; Molecular Biology of the Cell, Chapters 2 and 3, second edition, Eds. M. Robertson, R. Adams, Garland Publishing, NY.

20

Crustacea as used herein, refer to shellfish such as crab, shrimp, lobster, crawfish.

25

Crustacea tropomyosins used herein, refers to tropomyosin from shellfish as defined above.

30

Assays to assess an immunological change after the administration of the modified allergan are known to those skilled in the art. Standard IgE antibody tests include such conventional assays such as, for example, RAST (Sampson and Albergo, 1984), ELISAs (Burks et al., 1986) immunoblotting (Burks et al. 1988), and *in vivo* skin tests (Sampson and Albergo, 1984). Objective clinical symptoms can be monitored before and after the administration of the modified allergan to determine any change in clinical symptoms.

35

Reference herein to a "standard IgE antibody test" or related phrase means any one of the foregoing tests, particularly the IgE ELISA test, in which at least about 45%, preferably at least about 60%, more preferably at least about 75% of the binding between the crustacea tropomyosin and the IgE antibody has been reduced. Suitable controls for performing such a test are generally known in the field and include identified IgE antibodies that bind the tropomyosin and crustacea

40

- 5 tropomyosin e.g., that isolated from shrimp. More particular tropomyosins and IgE antibodies for performing the test are discussed below.

"Desensitization" as used herein is defined by a sufficient decrease in IgE antibodies, as measured by the above standard IgE tests, wherein the allergic
10 reaction of an individual manifests a decrease in clinical symptoms associated with exposure to an allergen, which can involve respiratory, gastrointestinal, skin, eyes, ears and mucosal surfaces in general.

A desensitizing amount as used herein, is a therapeutic composition of the
15 present invention employed in a physically discrete unit suitable as unitary dosages for a primate such as a human, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent or carrier. Precise desensitizing amounts of the therapeutic composition to be administered will be guided by the judgment of
20 the practitioner, however the unit dose will generally depend on the route of administration and be in the range of 10 ng/kg body weight to 50 mg/kg body weight per day, more typically in the range of 100 ng/kg body weight to about 10 mg/kg body weight per day.

25 Transgenic animals expressing the modified allergan have two purposes. First, they can be used as a source of modified allergan for use in immunotherapy and second, appropriately modified animals can be substituted for the original animal making immunotherapy unnecessary. Methods for the engineering of animals, for example, shrimp, crab, lobster or crawfish, are well known to those
30 skilled in the art. See for example, A. Colman, "Production of Therapeutic Proteins in the Milk of Transgenic Livestock" (1988) *Biochem. Soc. Symp.* 63:141-147 and Colman, *Am. J. Clin. Nutr.* 63(4):639S-645S, the teachings of which are incorporated herein.

35 It is important to administer the modified allergan to an individual (human or animal) to decrease the clinical symptoms of allergic disease by using a method, dosage, and carrier which are effective. The modified allergan will typically be administered in an appropriate carrier such as saline or a phosphate saline buffer. The modified allergan can be administered by injection subcutaneously,

- 5 intramuscularly, or intraperitoneally, by aerosol, inhaled powder, as a suppository, or by ingestion.

While one or more modified allergans of the invention may be administered alone, they may also be present as part of a pharmaceutical composition in mixture
10 with conventional excipient, preferably a pharmaceutically acceptable organic or inorganic carrier substances that is generally suitable for oral or nasal delivery. However, in some cases, other modes of administration may be indicated in which case the modified allergans, or combination of modified allergans thereof, can be combined with a vehicle suitable for parenteral, oral or other desired
15 administration and which do not deleteriously react with the modified allergans and are not deleterious to the recipient thereof. Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions, alcohol, vegetable oils, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and
20 diglycerides, petroethal fatty acid esters, hydroxymethyl-cellulose, polyvinylpyrrolidone, etc. The pharmaceutical preparations can be sterilized and if desired mixed with auxiliary agents, e.g. lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not
25 deleteriously react with the modified allergans.

For parenteral application, particularly suitable are solutions, preferably oily or aqueous solutions as well as suspensions, emulsions, or implants, including suppositories. Ampoules are convenient unit dosages.

30

For enteral application, particularly suitable are tablets, dragees or capsules having talc and/or carbohydrate carrier binder or the like, the carrier preferably being lactose and/or corn starch and/or potato starch. A syrup, elixir or the like can be used wherein a sweetened vehicle is employed. Sustained release
35 compositions can be formulated including those wherein the active component is protected with differentially degradable coatings, e.g., by microencapsulation, multiple coatings, etc.

It will be appreciated that the actual preferred amounts of modified
40 allergans, or combination of modified allergans, used in a given therapy will vary

5 according to the modified peptide or combination of peptides being utilized, the mode of application, the particular site of administration, etc. Optimal administration rates for a given protocol of administration can be readily ascertained by those skilled in the art.

10 The nucleotide molecule encoding the modified allergan can also be administered directly to the patient, for example, in a suitable expression vector such as a plasmid, which is injected directly into the muscle or dermis.

The modified allergan can be expressed by a vector containing a DNA
15 segment encoding the modified allergan.

These can include vectors, liposomes, naked DNA, adjuvant-assisted DNA, gene gun, catheters, etc. Vectors include chemical conjugates such as described in WO 93/04701, which has a targeting moiety (e.g. a ligand to a cellular surface
20 receptor), and a nucleic acid binding moiety (e.g. polylysine), viral vector (e.g. a DNA or RNA viral vector), fusion proteins such as described in PCT/US95/02140 WO 95/22618) which is a fusion protein containing a target moiety (e.g. an antibody specific for a target cell) and a nucleic acid binding moiety (e.g. a protamine), plasmids, phage etc. The vectors can be chromosomal, nonchromosomal or
25 synthetic.

Preferred vectors include viral vectors, fusion proteins and chemical conjugates. Retroviral vectors include moloney murine leukemia viruses. DNA viral vectors are preferred. Viral vectors can be chosen to introduce the modified
30 allergan to cells of choice. Such vectors include pox vectors such as orthopox or avipox vectors, herpesvirus vectors such as herpes simplex I virus (HSV) vector (Geller, A.I et al, J. Neurochem., 64:487(1995); Lim, F., et al., in DNA Cloning: Mammalian Systems, D. Glover, Ed. (Oxford Univ. Press, Oxford, England) (1995); Geller, A.I. et al., Proc. Natl. Acad. Sci. USA 87:1149 (1990)) Adenovirus vectors
35 (LeGal LaSalle et al., Science, 259:988 (1993); Davidson, et al., Nat. Genet. 3:219 (1993); Yang et al., J. Virol. 69:2004 (1995)) and Adeno-associated virus vectors (Kaplitt, M.G. et al., Nat. Genet. 8:148 (1994)).

Pox viral vectors introduce the gene into the cells cytoplasm. Avipox virus
40 vectors result in only short term expression of the nucleic acid. Adenovirus

5 vectors, adeno-associated virus vectors and herpes simplex virus vectors are preferred for introducing the nucleic acid into neural cells. The adenovirus vector results in a shorter term expression (about 2 months) than adenoassociated virus (about 4 months), which in turn is shorter than HSV vectors. The vectors can be introduced by standard techniques, e.g. infection, transfection, transduction or
10 transformation. Examples of modes of gene transfer include for example, naked DNA calcium phosphate precipitation, DEAE dextran, electroporation, protoplast fusion, lipofection, cell microinjection and viral vectors.

The vector can be employed to target essentially any desired target cell. For
15 example, stereotaxic injection can be used to direct the vectors (e.g. adenovirus, HSV) to a desired location. Other methods that can be used include catheters, intravenous, parenteral, intraperitoneal, and subcutaneous injection, and oral or other known routes of administration.

20 Another method is DNA immunization. DNA immunization employs the subcutaneous injection of a plasmid DNA (pDNA) vector encoding a specific allergenic protein. The pDNA sequence is taken up by antigen presenting cells (APC). Once inside the cell, the DNA encoding allergan is transcribed and translated. The allergan is then presumably presented on the surface of the APC in
25 the context of the major histocompatibility complex (MHC) to T-cells. This endogenously produced allergenic protein or protein fragment elicits a Th1 phenotypic response with up-regulation of IFN- γ , an increase in IL-2, and suppression of allergan-specific IgE production (Speigelberg HL, Orozco EM, Roman M, et al. *Allergy* 1997;52:964-70; Slater JE, Zhang YJ, Arthur-Smith A, et al., *J Allergy Clin Immunol* 1997;99:S504). Oral delivery of DNA immunizations has also
30 been described. Its been utilized as an immunoprophylactic strategy to modulate peanut antigen-induced anaphylaxis (Roy K, Mao HQ, Huang SK, Leong KW. *Nat. Med.* 1999;5(4):387-91). In this model, the oral delivery of DNA complexed to chitosan, a biocompatible polysaccharide, also favored a Th1 response and
35 suppressed the Th2 allergic immune response.

The vector pDNA can also be conjugated to immunostimulatory sequences (ISS). These ISS contain unmethylated cytosine and guanine dinucleotide repeat motifs. These CpG motifs stimulate APCs and natural killer cells to secrete IFN γ
40 and IL-12, cytokines that promote immune deviation toward the Th1 phenotype and

5 away from the allergic T_H2 phenotype (Chu RS, Targoni OS, Krieg AM, Lehman PV, Harding CV. *J. Exp. Med.* 1997;186:1623-31). These ISS stimulate immune deviation to the T_H1 phenotype when administered in several ways. They can be administered with DNA encoding the allergan (DNA immunization) (Hus C-H, Chua KY, Tao MH, Lai YL, Wu HD, Huang SK, et al., *Nat. Med.* 1996;2:540-4.), when
10 given alone (Bohle B, Jahn-Schmid B, Maurer D, Kraft D, Ebner C. *Eur. J. Immunol.* 1999;29:2344-53), or when conjugated with allergan. Although the majority of these DNA immunization techniques have been studied in the mouse model, Tighe and colleagues describe enhanced immune deviation to the T_H1 phenotype and reduced allergenicity after injection immunotherapy with the major
15 ragweed allergan, Amb a 1, conjugated to ISS in mice and rabbits, as well as in primates (Tighe H, Takabayashi K, Schwartz D, et al., *J. Allergy. Clin. Immunol.* 2000;106:124-34).

A vaccine as used herein, can include any of the above viruses or vectors
20 containing the entire nucleic acid sequence of the tropomyosin molecule, fragments thereof; modified nucleic acid sequences of the tropomyosin molecule; the entire amino acid sequence of the tropomyosin molecule or fragments thereof; modified amino acid fragments of the tropomyosin molecule or any peptides embodied in the invention.

25

The vaccine may be introduced in a suitable carrier. For example, sterile saline solution or sterile phosphate buffered saline.

In order to produce the desired therapeutic effect, as embodied in this
30 invention, the vaccine is desirably administered by subcutaneous or intramuscular injection. The treatment may consist of a single dose of vaccine or a plurality of doses over a period of time. An advantageous treatment schedule requires administration of two doses of vaccine with an interval of 3 to 7, preferably 4 to 6 weeks between doses. If longer protection is required, booster doses may be
35 administered after longer intervals, for instance after 6 months or annually. Those who are skilled in the art may modify the vaccine regimen according to the individual patient.

It is recommended that each dose is 0.5 to 5 ml, preferably 1 to 3 ml, most
40 preferably 2 ml of vaccine.

5

Antibodies of the invention can be prepared by techniques generally known in the art, and are typically generated to a purified crustacean tropomyosin molecule, to a modified crustacean tropomyosin molecule, preferably to peptides of a modified crustacean tropomyosin molecule or more preferably to modified peptide fragments thereof.

More particularly, antibodies can be prepared by immunizing a mammal with the above molecules, alone or complexed with a carrier. Suitable mammals include typical laboratory animals such as sheep, goats, rabbits, guinea pigs, rats and mice. Rats and mice, especially mice, are preferred for obtaining monoclonal antibodies. The antigen can be administered to the mammal by any number of suitable routes such as subcutaneous, intraperitoneal, intravenous, intramuscular or intracutaneous injection. The optimal immunizing interval, immunizing dose, etc. can vary within relatively wide ranges. Typical procedures involve injection of the antigen several times over a number of months. Antibodies are collected from serum of the immunized animal by standard techniques and screened to find antibodies specific for tropomyosin, modified tropomyosin, modified peptides of tropomyosin and fragments thereof. Monoclonal antibodies can be produced in cells which produce antibodies and those cells used to generate monoclonal antibodies by using standard fusion techniques for forming hybridoma cells. See G. Kohler, et al., *Nature*, 256:456 (1975). Typically this involves fusing an antibody producing cell with an immortal cell line such as a myeloma cell to produce the hybrid cell. Alternatively, monoclonal antibodies can be produced from cells by the method of Huse et al., *Science*, 256:1275 (1989).

30

One suitable protocol provides for intraperitoneal immunization of a mouse with a composition comprising the above-discussed antigens, conducted over a period of about two to seven months. Spleen cells can then be removed from the immunized mouse. Serum from the immunized mouse is assayed for titers of antibodies specific for the tropomyosin antigen selected, prior to excision of spleen cells. The excised spleen cells are then fused to an appropriate homogenic or heterogenic (preferably homogenic) lymphoid cell line having a marker such as hypoxanthine-guanine phosphoribosyltransferase deficiency (HGPRT) or thymidine kinase deficiency (TK). Preferably a myeloma cell is employed as the lymphoid cell line. Myeloma cells and spleen cells are mixed together, e.g. at a ratio of about 1 to

40

5 4 myeloma cells to spleen cells. The cells can be fused by polyethylene glycol (PEG) method. See G. Kohler, et al., *Nature, supra*. The thus cloned hybridoma is grown in a culture medium, e.g. RPMI-1640. See E. More, et al., *J. Amer. Med. Association*, 199:549 (1967). Hybridomas grown after the fusion procedure are screened such as by radioimmunoassay or enzyme immunoassay for secretion of
10 antibodies that bind to the above discussed tropomyosin antigens. Preferably an ELISA is employed for the screen. Hybridomas that show positive results upon such screening can be expanded and cloned by limiting dilution method. The isolated antibodies can be further purified by any suitable immunological technique including affinity chromatography.

15

Tropomyosin protein, including fragments and modified tropomyosins are often provide in substantially pure form. That is, the proteins have been isolated from cell constituents that naturally accompany it so that the proteins are present in at least 90 to 95% homogeneity (w/w). Proteins having at least 98 to 99%
20 homogeneity (w/w) are most preferred for many pharmaceutical, clinical and research applications including the vaccines disclosed herein. Once substantially purified the protein should be substantially free of contaminants for therapeutic applications. Once purified partially or to substantial purity, the proteins can be used therapeutically, or in performing *in vitro* or *in vivo* assays as disclosed herein.
25 Substantial purity can be determined by a variety of standard techniques such as chromatography and gel electrophoresis.

The tropomyosins in accord with the invention can be separated and purified by appropriate combination of known techniques. These methods include, for
30 example, methods utilizing solubility such as salt precipitation and solvent precipitation, methods utilizing the difference in molecular weight such as dialysis, ultra-filtration, gel-filtration, and SDS-polyacrylamide gel electrophoresis, methods utilizing a difference in electrical charge such as ion-exchange column chromatography, methods utilizing specific affinity such as affinity chromatograph,
35 methods utilizing a difference in hydrophobicity such as reverse-phase high performance liquid chromatograph and methods utilizing a difference in isoelectric point, such as isoelectric focusing electrophoresis, metal affinity columns such as Ni-NTA. See generally Sambrook et al. and Ausubel et al. *supra* for disclosure relating to these methods.

40

5 By the term specific binding or similar term is meant a molecule disclosed herein which binds another molecule, thereby forming a specific binding pair, but which does not recognize and bind to other molecules as determined by, e.g., Western blotting, ELISA, RIA, gel mobility shift assay, enzyme immunoassay, competitive assays, saturation assays or other suitable protein binding assays
10 known in the field.

The modified tropomyosins of the invention can be made by one or a combination of strategies. For example, nucleotide sequences encoding tropomyosin of crustacea or anthrop origin can be altered by mutations such as
15 substitutions, additions or deletions that provide for functionally equivalent nucleic acid sequence having reduced antigenicity (at the protein level). Preferred mutations are substitutions in the tropomyosin regions disclosed herein. In particular, a given nucleotide sequence can be mutated *in vitro* or *in vivo*, to create variations in coding regions and/or to form new restriction endonuclease sites or
20 destroy preexisting ones and thereby to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art can be used including, but not limited to, *in vitro* site-directed mutagenesis (Hutchinson et al., *J. Biol. Chem.*, 253:6551 (1978)), use of TAB Registered TM linkers (Pharmacia), PCR-directed mutagenesis, and the like. The functional equivalence of such mutagenized sequences, as
25 compared with unmutagenized sequences, can be empirically determined by comparisons of structural and/or functional characteristics.

The present invention is further illustrated by the following Examples. These Examples are provided to aid in the understanding of the invention and are
30 not construed as a limitation thereof.

EXAMPLES

Materials and Methods

Subjects' Sera

35 Sera collected from 18 atopic shrimp-allergic subjects were used to identify the IgE-binding regions of shrimp tropomyosin. All 18 subjects fulfilled four criteria: (1) history of respiratory (wheezing or shortness of breath), dermatologic (urticaria or angiodema), or gastrointestinal (nausea, vomiting, and/or diarrhea) symptoms occurring within 1 h following ingestion of shrimp; (2) positive immediate
40 skin prick test (SPT, wheal >3mm) to the cooked brown shrimp (*Penaeus aztecus*);

- 5 (3) elevated shrimp-specific IgE levels demonstrated by radioallergosorbent test (RAST, binding >3%) [24]; and (4) strong IgE reactivity to purified shrimp tropomyosin by immunoblot analysis. Three shrimp-allergic subjects without IgE reactivity to shrimp tropomyosin by immunoblot but fulfilling the other criteria for shrimp allergy were used as negative controls.

10

Shrimp extract and shrimp tropomyosin purification

Shrimp extract from locally purchased raw brown shrimp was prepared as described previously [24]. Pen a 1 was purified from shrimp extract by preparative SDS-PAGE (Model 491 PrepCell, Biorad). Briefly, shrimp extract was separated on
15 the 28 mm ID column using Laemmli discontinuous SDS-PAGE buffer system [25]. A 15 mm-high stacking gel (5%T, 1.5%C) poured on top of the 65 mm-high separation gel (11%T, 1.5%C) was used to separate shrimp proteins and the fractions containing Pen a 1 were collected and pooled.

20 IgE recognition of Pen a 1 by immunoblot analysis

Pen a 1 was run by SDS-PAGE [25] and transferred onto a CNBr-activated [26] nitrocellulose membrane (0.45 µm, BAS 45, Schneider and Schuell, Germany) at 0.8 mA/cm² for 30 min by semi-dry blotting [27]. The blots were blocked in TBS-Tween for 30 min, dried and stored between filter paper until use. Seventy five µl of
25 sera from each subject, diluted 1:2 in TBS-Tween (0.1M Tris HCl, pH 7.5, 0.1 M NaCl, 2.5 mM MgCl₂, 0.05% Tween) were incubated for 2 h with the Pen a 1-containing membrane using a Surfblot apparatus (Idea Scientific, Minneapolis MN). The membranes were washed in TBS-Tween and dried. To visualize IgE-binding proteins, the blot was incubated for 2 h with alkaline phosphatase
30 conjugated, monoclonal mouse anti-human IgE (Southern Biotechnology Associates, Birmingham AL) diluted 1:1000 in TBSTween and washed 3 times for 10 min in TBS-Tween. For detection of bound IgE, the membrane was washed 5 min in 37°C warm TBS-AP (0.1 M Tris-HCl, 0.1 M NaCl, 5 mM MgCl₂, pH 9.5) and antibody binding was visualized at using the substrate/chromogen mixture for
35 alkaline phosphatase at 37°C containing 450 µM 5-bromo-4-chloro-indolyl-phosphate disodium salt (BCIP; Sigma) and 400 µM nitroblue tetrazolium chloride (NBT; Sigma) solubilized in TBS-AP [28]. The reaction was stopped with deionized water and the blots were dried.

40 IgE antibody reactivity to synthetic overlapping Pen a 1 peptides: Peptide

5 **synthesis and IgE-binding assay**

Forty-six overlapping peptides were synthesized spanning the entire 284 amino acid residue length of Pen a 1. Each peptide had a length of 15 amino acid residues with an offset of six in relation to the previous and following peptides with the exception of peptide that had an offset of five amino acid residues (Figure 1).

10

Overlapping peptides were synthesized with Fluorenylmethoxycarbonyl (Fmoc) amino acids on cellulose membranes containing freehydroxyl groups according to the manufacturer's instructions of the SPOTS Epitope Mapping System (Genosys Biotechnologies, The Woodlands, TX). For the preparation of the Fmoc amino acid active ester solutions, each amino acid was dissolved in purified 1-methyl-2-pyrrolidinone (NMP 99%, Sigma-Aldrich, St. Louis, MO). Each amino acid solution was aliquoted and stored at -20°C until ready for use. Due to intrinsic instability, arginine was dissolved in NMP immediately prior to each synthesis cycle.

20

The procedure for peptide synthesis was performed as described previously [29-31]. Briefly, each cycle began by sterification of an Fmoc amino acid to the SPOTs cellulose membrane (Genosys Biotechnologies, Inc., The Woodlands, TX). Following incubation, the membranes were washed in N,N-didethylformamide (DMF, EM Science, Gibbstown, NJ) and 4% acetic anhydride in DMF was added to acetylate and block any uncoupled amino groups to prevent further reaction of these groups and formation of deletion sequences. After deprotection protective Fmoc groups were cleaved by incubation in 20% piperidine (Aldrich chemicals, Milwaukee, WI) in DMF, to render nascent peptides reactive. Each additional Fmoc amino acid was esterified to the previous one by the same process until the desired peptide is generated. After addition of the last amino acid, the protecting groups on the side chains of the amino acids were removed using a 1:1:0.05 mixture of dichloromethane (Aldrich)/ trifluoroacetic acid (TFA, Aldrich)/ triisobutylsilane (Aldrich), followed by washing in methanol. Membranes were either probed immediately or dried and stored at -20°C until needed.

35

For the IgE-binding assay, the membranes were first rinsed in methanol and washed in Tris buffered saline (TBS, pH 7.5) 3 times for 10min. The membranes were incubated in blocking solution (Genosys) diluted 1:10 in TBS for 2 h and then overnight with the patient's serum diluted 1:5 with blocking buffer. After washing three times for 15 min in TBS-Tween (TBS, 0.5% Tween; pH 7.5), IgE reactivities

40

- 5 were detected using 0.8 μ Ci per membrane of 125 I-labeled horse-anti human IgE (Sanofi Diagnostics Pasteur, Chasca, MN) diluted 1:10 in Genosys blocking solution. Next day, the membranes were washed 3 times for 15 min in TBSTween and placed between plastic sheets and exposed to X-ray film for 72 hours.
- 10 For interpretation of results, IgE reactivities were graded according to their intensity into four categories: negative (0), weak (1), medium (2) and strong (3), and color-coded as follows: negative (white), weak intensity (light gray), medium intensity (dark gray), and strong intensity (black). The intensity of the IgE reactivities was determined visually by agreement of 3 different investigators, who
- 15 graded the reactivities independently assigning the above scores.

Example 1

IgE binding regions of Pen a 1

- Table 1 shows the frequency and intensity of the IgE-recognition of the
- 20 different peptides by the 18 subject's sera. A wide range of peptides (from 1 to 16, mean of 8 per subject) were recognized by serum IgE of the shrimp-allergic, Pen a 1-reactive sera. In contrast, none of the three control sera from shrimp-allergic, Pen a 1-non reactive subjects showed IgE binding to any of the 46 peptides tested (data not shown). As an example, Figure 7 shows the peptides recognized by the serum
- 25 IgE of 6 shrimp-allergic subjects, with each spot representing a different peptide of all 46 tested. The number of subjects who recognized an individual peptide varied from 0 (0%) to 13 (72.2%). IgE-binding peptides were detected over most of the tropomyosin molecule. An intensity score (0-3) for each peptide was calculated by adding the individual scores obtained with the different sera (peptide score). The
- 30 mean intensity score of all peptides was obtained by adding all the peptide scores and dividing by 46 peptides (mean peptide score). A major IgE binding region was defined as a region recognized by serum IgE from more than 9/18 (50%) of the subjects, and/or when the score for intensity of IgE-binding of a particular peptide was larger than mean + 1 SDEV the mean peptide score (5.9 + 6.1).

35

- Based on frequency and intensity of the IgE reactivities, five major IgE binding regions were identified. All five major IgE-binding regions spanned from 1 to 4 peptides, with a length from 15 to 38 amino acid residues. Major IgE-binding regions identified were, region 1: Pen a 1 (43-57), region 2: Pen a 1 (85-105), region
- 40 3: Pen a 1 (133-148), region 4: Pen a 1 (187-202) and region 5: Pen a 1 (247-284).

5 Region 1 is recognized by 10/18 (55.5%) subjects, region 2 by 15/18 (83.3%), region 3 by 10/18 (55.5%), region 4 by 5/18 (27.5%) and region 5 by 12/18 (66.6%). The score for intensity of IgE recognition was 18, 19, 20, 12, and 12.2 for the five regions, respectively.

10 Example 2

Identified IgE-binding epitopes and epitope cores

Within regions 1, 2, 3 4 and 5 IgE-binding epitopes were identified using 5 to 9 amino acids-long peptides with an offset of two amino acids. If no or only minimal reactivity was detected the peptide size was increased to 9 to 15 amino acid
15 residues. The peptides that were found to be IgE-binding within regions 1 to 5 are also summarized in table 2.

Figures 3.1 to 3.5 show the identified strongest individual IgE-binding reactivities (epitopes) of regions 1 to 5, common epitope cores, and sequence
20 comparisons with allergenic and non-allergenic tropomyosins (Crustacea: *Penaeus aztecus* (brown shrimp, Pen a 1), *Metapenaeus ensis* (greasy-back shrimp, Met e 1), *Homarus americanus* (Atlantic lobster, Hom a 1), *H. americanus* slow muscle tropomyosin (HomaTMs), *Panulirus stimpsoni* (spiny lobster, Pan s 1). Insecta: *Periplaneta americana* (American cockroach, Per a 7), Arachnida: *Dermatophagoides pteronyssinus*, *D. farinae* (house dust mites, Der p 10, Der f 10). Vertebrata: *Gallus gallus* (chicken, alpha-tropomyosin Galg α TM; beta-tropomyosin Galg β TM), *Sus scrofa* (pig, Suss β TM), *Salmo trutta* (Atlantic salmon, SaltTM), *Oryctolagus cuniculus* (rabbit, Oryc β TM) (GenBank data)).

30 Region 1

All four subjects tested with peptides in region 1. For all for subjects the epitope was identical (Pen a 1 43-55, VHNLQKRMQQLEN) and 14 amino acids long. This sequence is identical to those from Met e 1, Hom a 1, and Pan s 1. However, slow lobster tropomyosin (HomaTMs) differs in 7 positions and vertebrate
35 tropomyosins differ in up to nine positions.

Region 2

Three of the four subjects tested reacted with Pen a 1 87-101 (ALNRRIQLLEEDLER), one subject reacted with a fraction of this sequence, Pen a 1
40 91-101 (RIQLLEEDLER), which is effectively the core of this epitope. This core

- 5 sequence is identical with those of other Crustacea and Per a 7, whereas Der p 10 carries one substitution in position 95. All vertebrate tropomyosins carry the same three substitutions in positions 95, 98, and 100.

Region 3

- 10 Six out of seven subjects tested reacted with peptides in region 3 (Pen a 1 133-146). The size of the individual epitope ranged from 6 to 9 amino acid residues. The core epitope of region 3 was Pen a 1 137-141 (DEERM) which is recognized by all 6 subjects and is identical to the homologous sequences of American cockroach (*Periplaneta americana*) and house dust mite (*Dermatophagoides pteronyssinus*)
15 allergenic tropomyosins, Per a 1 and Der p 10, respectively. The comparison with vertebrate tropomyosin shows a substitution in position 141 where an arginine (R) is substituted for a lysine (K) residue.

Region 4

- 20 All individual epitopes of the three subjects tested varied in length from 11 to 15 amino acid residues and begin with position 187. The shortest of the three epitopes (ESKIVELEEEL) is considered to be the epitope core. All homologous sequences of invertebrate tropomyosins are identical to the Pen a 1 sequence with the exception of Pan s 1 that carries a substitution in position 190. The vertebrate
25 tropomyosins differ from the homologous Pen a 1 sequence in up to four positions.

Region 5

- In region 5 (Pen a 1 247-284) three epitopes were identified. All four subjects reacted with Pen a 1 266-273 (KYKSITDE). This sequence was for all four subjects
30 the minimal IgE-binding site; no other peptide showed stronger reactivity. This epitope differs from both allergenic, arthropod and non-allergenic, vertebrate tropomyosins. The second epitope of region 5, is centered around a core, Pen a 1 251-259 and is recognized by 3/4 subjects; the core does not differ for the homologous sequences of Per a 1 or Der p 10 but differs in three positions from
35 non-allergenic, vertebrate tropomyosins. Two of 3 individually recognized epitopes were larger (12 residues) than the average epitope size (9 residues). The third epitope in region 5 (Pen a 1 273-281) is only recognized by one subject and does not seem to be an important epitope.

- 40 The Examples shown above demonstrate that individual epitopes have large

5 overlaps and are clustered around a common core sequences. The identity of some of these epitopes cores with homologous, allergenic tropomyosins of American cockroach (*P. americana*) and house dust mite (*D. pteronyssinus*) tropomyosin may explain reported cross-reactivities between shrimp and other arthropods. The limited amino acid sequence differences between individual epitopes as well as
 10 epitope cores and non-allergenic tropomyosins makes it possible to test all possible combinations of amino acid substitutions (see below)

Example 3

Combinatorial Substitution Analysis

15 In order to reduce/abolish the antibody reactivity, the individual IgE-binding peptides (= epitopes) of regions 1, 2, 3, and 4 are gradually mutated into the homologous sequences of invertebrate (allergenic) and vertebrate (non-allergenic) tropomyosins (figure 4). Individual sera were tested for IgE reactivities to these peptides. The reactivities were scored according to their intensities and color-coded
 20 (minimal, moderate, strong, very strong). The sequences of and the reactivities to these peptides are listed in the following tables 3.1, 3.2., 3.3., 3.5, and 3.5. The corresponding autoradiographs are shown in figures 5.1, 5.2., 5.3., and 5.4, respectively.

25 The aim of these experiments was to study the effects of amino acid substitutions on the IgE binding capacity of an allergenic epitope if a nonallergenic homologous protein is not known, homologous sequences of allergenic, invertebrate and non-allergenic, vertebrate tropomyosins served as templates for the substitutions. The following muscle tropomyosins were used as templates
 30 (GenBank data):

	Crustacea:	<i>Homarus americanus</i> (Atlantic lobster, Hom a 1)
		<i>H. americanus</i> slow muscle tropomyosin (HomaTMs)
		<i>Panulirus stimpsoni</i> (spiny lobster, Pan s 1)
	Insecta:	<i>Periplaneta americana</i> (American cockroach, Per a 7)
35	Arachnida:	<i>Dermatophagoides pteronyssinus</i> , (house dust mite, Der p 10).
		<i>D. farinae</i> (house dust mite, Der f 10)
	Vertebrata:	<i>Gallus gallus</i> (chicken alpha-tropomyosin, Gal α TM)
		<i>G. gallus</i> (chicken beta-tropomyosin, chicken Gal β TM),
		<i>Sus scrofa</i> (swine alpha tropomyosin, Sus α TM),
40		<i>Salmo trutta</i> (Atlantic salmon, SaltTM1, SaltTM2)

- 5 *Oryctolagus cuniculus* (rabbit alpha-tropomyosin, Ory α TM)
O. cuniculus (rabbit beta-tropomyosin, Ory β TM)

Due to the limited number of possible substitutions, the combinatorial substitution analysis was performed with individual sera rather than serum pool.

10 The substitutions and their effect on the IgE-binding capability of modified Pen a 1 peptides can be categorized according to different criteria. First, the minimal number of substitutions that a peptide must carry to render it nonreactive and second, the maximal number of substitutions per peptide allow the peptide to retain at least some IgE reactivity. In all 17 epitope-subject combinations that were

15 studied by mutational analysis, one substitution may be sufficient to render Pen a 1 epitopes non-IgE binding. However, this is the case in only 57.6% of the 170 peptides tested that carried a single substitution; the remaining 42.4% of the peptides carrying a single substitution still bind IgE antibodies. 82.4% of peptides that differ from the homologous Pen a 1 sequence in two positions are still able to

20 bind IgE antibodies. In general for most (99.9%) peptides that carry three or more substitutions do not show any IgE antibody reactivity; only one peptide carrying three substitutions bond IgE antibodies.

Critical Amino Acid Positions

25 The mutated peptides were analyzed in regard to positions within individual epitopes that were "critical" for IgE antibody binding. A critical position was defined as a position that, when substituted, abolished the IgE antibody reactivity of all peptides that contained this mutation. The results of this analysis is summarized in figure 6; it shows the critical amino acid positions (marked in **figure 6**) for each

30 individually epitopes within regions 1, 2, 3, and 5. Furthermore, figure 6 gives the amino acid that has to be substituted into these critical positions to abolish IgE antibody binding.

Table 5 lists all amino acid substitutions in Pen a 1 epitopes 1, 2, 3a, 3b, 5a, 35 5b, and 5c that result in complete loss or reduction of IgE antibody reactivity. The first column lists all the sequences that carry substitutions that abolished IgE antibody reactivity with any of the sera that were used to analyze that particular epitope. The second column lists all the sequences that carry substitutions which in some cases reduce or abolish IgE antibody reactivity with the sera that were

40 used to analyze the previously unaltered Pen a 1 epitopes. The sequences are

5 named according to the substitutions they carry (King et al., Allergan
Nomenclature. WHO/IUIS Allergan Nomenclature Subcommittee *Int. Arch. Allergy
Immunol.* 1994, 105(3):224-233). For example, Pen a 1 44 is a Pen a 1 sequence
that has an I (Isoleucine) in Pen a 1 position 44. This Isoleucine replaces a H
(Histidine) that can be found in position 44 in the unaltered Pen a 1 sequence (see
10 row labeled as "Pen a 1 sequence").

Critical Amino Acid Positions of Epitopes of Region 1

No critical amino acid position was identified within epitopes of region 1.

15 Critical Amino Acid Positions of Epitopes of Region 2

Within the epitopes of region 2, three critical amino acid positions (95, 98,
100) were identified. Of these three positions, position 95 seems to be the most
crucial, since a substitution of Leucine (L) with Phenylalanine (F) abolished the IgE
antibody reactivity of all three subjects' sera tested.

20

Critical Amino Acid Positions of Epitopes of Region 3

To Abolish the IgE antibody reactivity of individual 3a epitopes, mutations in
two critical positions are necessary. The first critical position is located at position
142 and an Aspartate (D) is replaced with Glutamate (E); it abolished the IgE
25 antibody reactivity of four of the five epitope 3a-reactive sera. The second critical
position is position 136; a Serine (S) has to be replaced with a Lysine (K) to abolish
the IgE antibody reactivity of three subjects' sera.

The critical position of epitope 3b is position 144; a mutation from a Leucine
30 (L) to a Glutamine (Q) abolished the IgE antibody reactivity of both subjects' sera
tested.

Critical Amino Acid Positions of Epitopes of Region 5

Within the 5a epitopes, four critical amino acid positions (250, 252, 255,
35 260) were identified. Of these four positions, position 255 seems to be the most
crucial, since a substitution of Arginine (R) with Aspartate (D) abolished the IgE
antibody reactivity of all four subjects' sera tested. Similarly, a substitution (Serine
(S) in Phenylalanine (F)) in the one critical position 269 of epitope 5b rendered all
peptides containing this substitution non-IgE-binding. Epitope 5c had four critical
40 positions (277, 278, 280, 281); however, since only one subject reacted to this

5 epitope, it is not possible to identify the most crucial substitution.

Example 4

Amino acid composition of the IgE-binding regions

To determine if certain groups of amino acids may be important for IgE
10 binding, the relative frequency of different amino acids in the tropomyosin molecule
relative to the main IgE-binding regions were analyzed. Five categories of amino
acids were considered: non-polar, aliphatic (alanine A, glycine G, isoleucine I,
leucine L, proline P, valine V; polar, uncharged (cysteine C, asparagine N,
methionine M, glutamine Q, serine S, threonine T; aromatic (phenylalanine F,
15 tryptophane W, tyrosine Y); positively charged (histidine H, lysine K, arginine R)
and negatively charged (aspartic acid D, glutamic acid E). The frequency of the
different groups of amino acids in each IgE-binding region was considered in
relation to the number of amino acids present in that group, supposedly that all
amino acids have the same probability to appear in a protein (probability 1). For
20 the whole molecule, negatively charged amino acids are 2.5 times more frequent
than would be expected by chance. Contrarily, aromatic residues are almost absent
in the tropomyosin molecule. The other three groups of amino acids are present in
the molecule with the frequency expected considering all 20 amino acids equally
probable. In the five main IgE-binding regions the frequency of each amino acid
25 group is the same as the frequency observed in the whole molecule. No substantial
differences in amino acid group composition in the five IgE-binding regions
compared to the whole molecule were detected.

When individual amino acids were analyzed, it was noted that several amino
30 acids such as proline, cysteine and tryptophane were absent from the molecule and
others such as glycine, isoleucine, tyrosine and histidine were rarely found. In
contrast, alanine, leucine, lysine, arginine, and glutamic acid were present in the
molecule at least 2 times more frequent than expected by chance. Only alanine
seems to be less frequent in the IgE-binding regions than in the rest of the
35 molecule. No differences in respect to the distribution of amino acids in the
tropomyosin molecule were noticed, since all other amino acids are similarly
represented in Pen a 1.

Example 5

Allergenic motifs

5 In previous studies, the presence of tandem amino acid repeats contained within allergens has been reported [32, 33]. In our study, all five major IgE-binding regions identified within Pen a 1 contain the amino acid sequence LEXXL, where L is Leucine and X is usually a negatively charged amino acid such as glutamic acid (E) or aspartic acid (D). In regions 1 and 3, X may be an aspartic acid (D),
 10 glutamine (Q) or asparagine (N). The tandem motifs occupy positions 53-57 (motif 1), 95-99 (motif 2), 144-148 (motif 3), 193-197 (motif 4) and 256-260 (motif 5). Even though IgE reactivities were detected to Pen a 1 peptides which did not contain the tandem repeat, every tandem amino acid repeat was found to be included within a major Pen a 1 IgE-binding region.

15

Example 6

Sequence identity and similarity of Pen a 1 and Pen a 1 IgE-binding regions with homologous regions in other allergenic and nonallergenic tropomyosins

Sequence identities (% of amino acids identical in both compared
 20 tropomyosins) and similarities (% of amino acids identical or belonging to the same amino acid group) in the amino acid sequences of different tropomyosins with Pen a 1 IgE-binding regions are represented in Table 4. Sequence identity among Pen a 1 and other tropomyosins varied from 56% (rabbit tropomyosin) to 98% (lobster fast tropomyosin); similarity in amino acid composition ranged from 72% to 98%
 25. respectively, with the highest similarities (over 80%) being observed within arthropods (insects, arachnids and crustaceans). Remarkably amino acid sequences of several IgE binding regions of Pen a 1 showed significant degree of identity with homologous sequences of other tropomyosins, specially among arthropods such as lobster, house dust mites, cockroach, fruit fly, reaching up to
 30 100% identity in particular sequences (Table 4).

Similarities of Pen a 1 regions with tropomyosin of mollusks such as mussels, helminths such as *Onchocerca*, and surprisingly, also with vertebrates such as rabbit, although varied depending on the regions considered, were as high
 35 as 86%, 94% and 85% respectively. Region 1 is identical within crustaceans but differs from other invertebrates and vertebrates, with only 26%, 60% or 33% identity with *Schistosoma*, insects and vertebrates respectively. Interestingly, regions 2 and 4 show 100% similarity with homologous regions of arthropod tropomyosins from American cockroach *Periplaneta americana* (Per a 7), fruit fly *Drosophila*
 40 *melanogaster* (Dro m TM), house dust mites *Dermatophagoides pteronyssinus* and

5 *D. farinae* (Der p 10, Der f 10). Furthermore, regions 2 and 4 show significant
similarity with corresponding sequences in other tropomyosins, including those
from vertebrates (up to 85 %). Regions 3 and 5 are identical within crustaceans,
and the identity with tropomyosins of other arthropods reaches up to 89%. In
10 contrast, these Pen a 1 regions differ substantially from those of homologous
sequences (as low as 40% identity) in helminths and vertebrate tropomyosins.

Example 7

Comparison of IgE-binding regions identified in Pen a 1 and in other allergenic tropomyosins.

15 Even though many invertebrate tropomyosins have been identified as
allergenic, the location of the IgE-binding epitopes has been partially identified for
only a small number of them. Figure 8 shows the amino acid sequence comparison
of Pen a 1 with other allergenic tropomyosins, whose B cell epitopes have been
partially characterized such as Pen i 1 from the shrimp *Penaeus indicus*, Tur c 1
20 from the snail *Turbo cornutus*, and Cra g 1 from the oyster *Crassostrea gigas*.
Results from a previous study by our group of IgE binding epitopes from Pen a 1
using a recombinant peptide library of Pen a 1 are also included (regions E2, E3,
E4 and E6). Most of the 5 IgE binding regions identified in Pen a 1 in the current
study partially or completely overlap with those observed in other allergenic
25 tropomyosins. Of particular importance seems to be region 5, identified also as an
important IgE-binding region in the snail tropomyosin, Tur c 1, since the strongest
IgE reactivities of both shrimp-allergic and snail-allergic individuals were directed
towards this region.

30 Over the last years, the primary structures of a number of allergans have
been characterized and T and B cell epitopes of several allergans have been
identified. Knowledge of the B-cell and T-cell epitopes in food allergans is important
because this could serve as the basis for the development of new safer peptides for
food allergy immunotherapy, as well as for the introduction of new genetically
35 modified hypoallergenic foods. Although not yet fully established, immunotherapy
with T-cell peptides of some allergans has been demonstrated in a few cases to be
effective. For instance, peptide fragments containing T-cell epitopes of bee venom
phospholipase A2 intact, whose B-cell epitopes have been modified to abolish IgE
binding, have been successfully used for immunotherapy with a lower risk of
40 reactions [34]. Therefore, identification and subsequent modification of the Bcell

- 5 epitopes of tropomyosin could serve as the basis for the development of new safer immunotherapy for food allergy, and as well as for the introduction of new hypoallergenic foods.

10 In this study, five major Pen a 1 IgE-binding regions have been identified using synthetic overlapping peptides, 15 amino acids long with offset of 6, spanning the whole length of Pen a 1. The five IgE-binding regions are distributed along the molecule at approximately every 42 amino acid residues. This results suggest a relation with the heptameric repeat pattern characteristic for the - helical, coiled-coil structure of tropomyosin [21]. The five regions identified contain
15 at least 15 amino acid residues (region 5 spans 37 residues). Since other studies have determined the IgE binding epitopes of other allergens to consist of approximately 8 amino acids [29-31, 33], most probably the identified IgE-binding regions of Pen a 1 are larger than their epitopes and each region may contain several epitopes. The smallest IgE-binding sequences within these regions of Pen a
20 1 remain to be investigated.

Previous studies of the homologous cockroach allergens Per a 1 and Bla g 1 [32] and the latex allergen Hev b 5 [33], have reported the presence of tandem amino acid repeats within allergens. Per a 1 and Bla g 1 sequences contain
25 multiple tandem amino acid repeats of approximately 100 amino acid residues. Hev b 5, presents 9 repeated amino acid sequences of the type XEEEX or XEEEX; four peptides of 8 amino acids containing the sequence XEEEX were found to be IgE binding epitopes. In contrast, none of the peptides containing the motif XEEEX bound IgE from latex-allergic subjects. In our study, all five major IgE-binding
30 regions identified within Pen a 1 contain the amino acid sequence LEXXL. Even though IgE reactivity to other Pen a 1 peptides that do not contain the tandem repeat was detected, every repetition of the tandem motif was found to be included within a major Pen a 1 IgE-binding region.

35 Shrimp-allergic patients may react to other crustaceans and sometimes to mollusks [24, 35], and substantial in vitro cross-reactivity among crustaceans has been demonstrated. Furthermore, allergenic cross-reactivity between arachnids (house dust mite), crustaceans (shrimp) and mollusks (snails, limpet) has been suggested to be of clinical relevance, especially in subjects receiving mite
40 immunotherapy [17, 19]. Also, it has been reported that some patients may become

- 5 mite and/or cockroach-allergic subsequently to their sensitization to crustacean tropomyosin due to unusual ingestion of crabs and shrimp.

Comparison of the amino acid sequence of the entire Pen a 1 molecule with different tropomyosins showed that the identity ranged from 56% (rabbit
10 tropomyosin) to 98% (lobster fast muscle tropomyosin). Similarities were even higher when conservative substitutions were not considered as different amino acids; the highest similarities were found among arthropods which reached over 80%. When the amino acid sequences within Pen a 1 IgE binding regions were compared with corresponding areas in other tropomyosins, the degree of similarity
15 was found to be remarkably high; 100% identity of all five regions with the major lobster allergen Hom a 1 (fast muscle tropomyosin) was detected explaining the high degree of cross-reactivity among crustaceans. Sequence identity of Pen a 1 IgE binding regions with Per a 7 and Der p 10 is very high (60-100%) which might suggest similar IgE-binding epitopes in arthropods. Also consistent with this
20 observation is the identity of Pen a 1 regions 2 and 4 with homologous amino acid sequences of tropomyosin from *D. melanogaster*. Similarities of Pen a 1 regions with those from mollusks and helminths are lower, but still as high as 86 and 94%, respectively. The importance of the cross-reactivity with helminths has been far less studied, but also reported [36].

25 Furthermore, comparison of IgE-binding regions identified in Pen a 1 and in other allergenic tropomyosins support our results. Results from a previous study by our group of IgE binding epitopes from Pen a 1 using a recombinant library are also included (regions E2 [167-179], E3 [136-148], E4 [262-282] and E6 [157-169])
30 [37]. Most of the five IgE-binding regions identified in Pen a 1 in the current study partially or completely overlap with those observed in other allergenic tropomyosins. Regions 2 and 5 seem to be of particular importance, since the homologous sequences in oyster (Cra g 1) and in the snail (Tur c 1) tropomyosins bind IgE antibodies of mollusk-allergic subjects, thus supporting the notion that
35 tropomyosin is the cause of clinically relevant cross-sensitization between crustaceans and mollusks [24, 35].

The Examples further show *in vitro* cross-reactivity among tropomyosins from different invertebrates on the molecular level and demonstrates the
40 importance of protein structure and its relationship to their allergenicity.

5

Example 8**Characterization and Identification of Allergan Epitopes: R combinant Peptide Libraries and Synthetic, Overlapping Peptides.**10 **Material and Methods****Recombinant Peptide Library**

In order to characterize Pen a 1 epitopes, a recombinant peptide library (Novatope epitope mapping system, Novagen) was constructed. The Pen a 1 coding plasmid was randomly cleaved by DNase I in the presence of Mn^{2+} ; causing double strand cleavage. Electrophoretically separated fragments, averaging 50 to 150 bp in size, were eluted (QIAEX II Agarose Gel Extraction Kit, Qiagen), treated successively with T4 DNA polymerase and Tth DNA polymerase, ligated into the pTOPE T vector, and transfected into NovaBlue (DE3) cells. The library was screened with a sera pool of shrimp-allergic subjects and positive clones sequenced.

20

Synthetic, Overlapping Peptides

Forty-six overlapping peptides (length: 15 amino acids, offset: six amino acids, figure 1) spanning the entire Pen a 1 molecule, were synthesized using the SPOTs system (Genosys, The Woodlands, TX). The SPOTs system cellulose membrane contains 96 blue spots which are derivatized with a dimer of balanine-NH₂ groups that provide six atom linkers (anchor) between the membrane and the peptide. Fmoc-OPfp esters of the amino acids are coupled by repeated amino acid pipetting and washing. Amino acids are linked together by a condensation reaction between the C-terminal and N-terminal groups of two amino acids in a C-terminal to N-terminal direction from the membrane. The coupling reaction is monitored visually by staining the free amines after each coupling cycle with bromophenol blue. The resulting peptides are covalently bound to the membrane at their C-terminus. Each synthesis cycle begins with esterifying the appropriate Fmoc amino acid to the cellulose membrane or the previous amino acid. The coupling reactions are followed by acetylation with acetic anhydride in N,N-dimethylformamide to render the peptides unreactive during subsequent cycles. The Fmoc protective groups are removed by adding piperidine to activate the nascent peptides. To add the remaining amino acids the same cycle of coupling, blocking, and deprotection is repeated until the desired peptides are generated. The side chains are then

deprotected with a 20:20:1 mixture of dichloromethane, trifluoroacetic acid, and

5 triisobutylsilane and washed with methanol. The membranes are stored at -20°C until used. The synthesis schedules can be calculated using the software provided by Genosys or using the graphing calculator HP48GX. The advantage of the HP48GX printouts is that the positions of a particular amino acid are provided as graphs rather than lists of position numbers.

10

For immunodetection, the membranes were blocked and incubated with 1:5 diluted serum pool or individual sera of shrimp-allergic subjects overnight. IgE reactivities were detected, either using 10 ml ¹²⁵I-labeled horse-anti-IgE (0.08 µCi/ml; Sanofi Diagnostics Pasteur, Inc.) or monoclonal alkaline phosphatase-labeled anti-human-IgE (Southern Biotechnology Associates, Birmingham, AL, USA) and autoradiography. The exposure time for ¹²⁵I-labeled anti-IgE was 72 h. For the detection of IgE antibodies using the alkaline phosphatase-conjugated monoclonal antibodies blots were washed with freshly prepared assay buffer (100 mM diethanolamine/HCl, 1.0 mM MgCl₂ pH 10.0), incubated in 1:50 diluted . Nitroblock[®] chemiluminescence enhancer (Tropix, Bedford, MA) for 5 min and incubated in a 1:1000 dilution of CSPD (disodium 3-(4-methoxy-spiro(dioxetane-3, 2'-(5'-chloro) tricyclo[3.3.1.1.1^{3,7}]decan)-4-yl)phenyl phosphate; Tropix) for 5 min. Excessive liquid was drained, and the blots were sealed between transparencies and exposed to autoradiography film for 15, 30, 60 and 120 sec.

25

Comparison IgE reactivities to Recombinant and Synthetic, Overlapping Peptides

Four recombinant peptides and nine synthetic peptides bound Pen a 1-specific IgE, respectively (Table 6) and are located at the N-terminus, center, and C-terminus of the Pen a 1 molecule. No reactivity was detected in the N-terminal part of Pen a 1 using the recombinant peptide library. In general, the SPOTs system detects more IgE-binding sequences. However, two IgE-binding sequences, Pen a 1 157-169 and Pen a 1 167-179 that were detected using the recombinant peptide library were not detected using synthetic, overlapping peptides even though the entire sequence Pen a 1 157-169 is part of a synthetic peptide. In contrast Pen a 1 167-179 is part of two overlapping peptides (figure 9).

Table 7 shows all the substitutions (position, pos; substituting amino acid, aa) that can be considered to reduce or abolish the IgE reactivity of major and minor IgE-binding regions. In addition to the five major regions, minor IgE-reactive

- 5 regions, defined as regions to which at least one allergic subject shows strong IgE antibody reactivity.

The Example shows that the methods may be used to identify IgE-binding sequences of food allergans, and the SPOTs procedure resulted in the identification of more epitopes of the major shrimp allergan Pen a 1. However, since the sequences of the synthetic, overlapping peptides have a defined offset, epitopes that are located on two peptides overlapping may not always be readily found. In regard to IgE detection, the usage of ¹²⁵I-labeled detection antibody seems to be superior over enzyme-labeled anti IgE antibodies. The regeneration of SPOTs membranes is possible, but it is prudent to test regenerated membranes for residual activity. We synthesize new peptides for each experiment. Besides the technical aspects other factors may influence the technique used for epitope identification of a given food allergan. First, to create a recombinant peptide library, it is necessary to have an expressed full-length allergan or fragments that span the entire length of the allergan and have significant overlaps. The sequences for synthetic, overlapping peptides can be deduced from information available through data bases such as GenBank or SwissProt. Second, it is not possible to ensure that entire allergan sequence is represented in the peptide library whereas overlapping peptides guarantee systematic coverage of the entire allergan sequence. Third, an advantage of the recombinant library method is that the peptide length is not limited to 15 residues it is the case for the SPOTs system which may allow the identification of at least some conformational epitopes. An additional advantage of the Novatope system is that it is easy to test additional patients' sera by simply growing more peptide-expressing *E.coli* and use lysates in a dot blot or grid blot. Synthetic peptides have to be resynthesized which requires in comparison a much higher experimental effort. Fifth, a major advantage of synthetic peptides is the ease in which the impact of amino acid substitutions have on the IgE binding of epitopes [38,39,40]; the side-by-side comparison of unmodified and mutated epitopes allows an easy quantification of changes of protein structure on the allergenicity of proteins [39]. This approach may be used to produce foods and other allergans with reduced allergenicity.

Since the synthesis conditions are not optimized for each amino acid or peptide the question arises whether the synthesized peptides have the correct sequence. The synthesis protocol uses an acetylation step at the end of each cycle

5 to acetylate any unreacted free amines with acetic anhydride. This prevents them
from coupling to any subsequent amino acids and virtually eliminates the synthesis
of deletion sequences. The purity of the peptides synthesized varies for each peptide
and is dependent upon sequence and length even though the peptide purity is
typically larger than 70% (Genosys, personal communication). As a consequence it
10 is essential to verify the results obtained with overlapping peptides with highly
purified peptides when peptides are designed for critical applications such as
allergen-specific immunotherapy.

To summarize the foregoing results, if a given food allergen contains
15 significant linear epitopes, which seems to be true for stable major allergens such
as those of peanut and shrimp the SPOTs system may be more advantageous than
the use of recombinant peptides libraries. However, if allergens are studied that
contain more conformational epitopes, recombinant peptide libraries may help to
identify the relevant epitopes.

20

Example 9

Immunotherapy

DNA sequences coding for modified and unmodified Pen a 1, Pen a 1
fragments, or Pen a 1 peptides will be administered subcutaneously,
25 intramuscular, or orally. Dosage, frequency and duration of treatment will be
adjusted on an individual basis. DNA immunization will employ naked DNA,
plasmid DNA (pDNA) vectors encoding for modified and unmodified Pen a 1, Pen a
1 fragments, or Pen a 1 peptides as well as pDNA conjugated to immunostimulatory
sequences (ISS). These ISS contain unmethylated cytosine and guanine
30 dinucleotide repeat motifs. These
CpG motifs and DNA molecules stimulate antigen-presenting cells and natural
killer cells to secrete IFN- γ and IL-12, cytokines that promote immune deviation of
T lymphocytes toward the Th1 phenotype, away from the allergic Th2 phenotype,
and, thus reduce the production of allergen-specific IgE antibodies. These DNA
35 molecules, plasmid DNA and ISS will be administered in several ways. They can be
administered with DNA encoding the allergen (DNA immunization), given alone, or
conjugated with modified and unmodified allergen, allergen fragments, and
peptides.

40 Example 10

5 **Antibodies specific for modified allergens**

Modified and unmodified Pen a 1, Pen a 1 fragments, or Pen a 1 peptides will be used to produce specific antibodies in mice, rats, rabbits or other experimental animals. The produced sera and monoclonal antibodies will be used to detect, measure and standardize modified and unmodified Pen a 1, Pen a 1 fragments, or
10 Pen a 1 peptides, that are produced for diagnostic and therapeutic purposes. In this context, monoclonal antibodies are especially useful as secondary standards, since monoclonal antibodies recognize only specific determinants (epitopes) of allergens and non-allergens; the specificity of an antibody depends on the uniqueness of the epitope. Furthermore, these antibodies and antisera will also be
15 very useful to characterize cross-reacting epitopes on related and unrelated proteins.

Example 11

Transgenic crustacea

20 The DNA coding for the mutated tropomyosins will be developed by site-directed mutagenesis of Pen a 1. Using the spermatophore-microinjection (SMI) technique (Li SS, Tsai HJ Transfer of foreign gene to giant freshwater prawn (*Macrobrachium rosenbergii*) by spermatophore-microinjection (SMI). Mol. Reprod. Dev. (2000) Jun;56(2):149-154) the mutated Pen a 1 will introduced into the
25 genome of shrimp or prawn species such as the giant freshwater prawn *Macrobrachium rosenbergii* or Tiger shrimp (*Penaeus monodon*). Approximately 1 µg of the circular plasmid DNA will be directly microinjected into spermatophores. Fertilization and hatching of shrimp or prawns created with SMI were completed in vivo. The genomes of free swimming, SMI-created larvae (21 days after fertilization)
30 will be analyzed by PCR and Southern blot analyses.

It will be apparent that any of the modified tropomyosins disclosed herein including particular modified shrimp tropomyosins can be transduced into shrimp to make the transgenic shrimp. Such shrimp may or may not include tropomyosin
35 "knock-out" mutations as needed for a particular application. Such transgenic shrimp will be especially useful in seafood farming applications featuring shrimp with reduced or negligible allergen potential.

The invention has been described in detail with reference to preferred
40 embodiments thereof. However, it will be appreciated that, upon consideration of

- 5 the present specification and drawings, those skilled in the art may make modifications and improvements within the spirit and scope of this invention as defined by the claims.

The following references are cited throughout the specification. All
 10 documents mentioned herein, including the references listed below, are incorporated by reference.

1. FDA. Report of the FDA ad Hoc committee on hypersensitivity to food constituents. U.S. Food and Drug Administration, Washington, D.C. 1996.
- 15 2. Taylor SL, Bush RK. Allergy by ingestion of seafoods. In: ATT., ed. Marine Toxins and Venoms. New York: Marcel Dekker, Inc., 1988;3:149-183.
3. Leung PS, Chu KH, Chow WK, et al. J Allergy Clin Immunol 1994;94(5):882-890.
4. Shanti KN, Martin BM, Nagpal S, Metcalfe DD, Rao PV. J Immunol
 20 1993;151(10):5354-5363.
5. Daul CB, Slattery M, Reese G, Lehrer SB. Int Arch Allergy Immunol 1994;105(1):49-55.
6. Mykles DL, Cotton JL, Taniguchi H, Sano K, Maeda Y. J Muscle Res Cell Motil 1998;19(2):105-115.
- 25 7. Leung PS, Chen YC, Mykles DL, Chow WK, Li CP, Chu KH. Molecular Marine Biology & Biotechnology 1998;7(1):12-20.
8. Leung PS, Chen YC, Gershwin ME, Wong SH, Kwan HS, Chu KH. J Allergy Clin Immunol 1998;102(5):847-852.
9. Miyazawa H, Fukamachi H, Inagaki Y, et al. J Allergy Clin Immunol
 30 1996;98(5 Pt 1):948-953.
10. Ishikawa M, Ishida M, Shimakura K, Nagashima Y, Shiomi K. Bioscience, Biotechnology & Biochemistry 1998;62(7):1337-1343.
11. Ishikawa M, Ishida M, Shimakura K, Nagashima Y, Shiomi K. J Food Science 1998;63:44-47.
- 35 12. Aki T, Kodama T, Fujikawa A, et al. J Allergy Clin Immunol 1995;96(1):74-83.
13. Asturias JA, Arilla MC, Gomez-Bayon N, Martinez A, Martinez J, Palacios R. Biochimica et Biophysica Acta 1998;1397(1):27-30.
- 40 14. Asturias JA, Gomez-Bayon N, Arilla MC, et al. Journal of Immunology 1999;162(7):4342-4348.

- 5 15. Santos AB, Chapman MD, Aalberse RC, et al. *J Allergy Clin Immunol* 1999;104(2 Pt 1):329-337.
16. Martinez A, Martinez J, Palacios R, Panzani R. *Allergol Immunopathol (Madr)* 1997;25(3):118-126.
17. van Ree R, Antonicelli L, Akkerdaas JH, Garritani MS, Aalberse RC, Bonifazi
10 F. *Allergy* 1996;51(2):108-113.
18. Carrillo T, Rodriguez de Castro F, Blanco C, Castillo R, Quiralte J, Cuevas M. *Ann Allergy* 1994;73(6):504-508.
19. Banzet ML, Adessi B, Vuitton DA, L'Amecoforcal. *Rev Fr Allergol* 1992;32:198-202.
- 15 20. Reese G, Ayuso R, Lehrer SB. *Int Arch Allergy Immunol* 1999;119(4):247-258.
21. Smillie LB. *Trends in Biochemical Sciences* 1979;4:151-155.
22. Restani P, Fiocchi A, Beretta B, Velona T, Giovannini M, Galli CL. *J Am Coll Nutr* 1997;16(4):383-389.
- 20 23. Ayuso R, Lehrer SB, Lopez M, et al. *Allergy* 2000;55(4):348-354.
24. Lehrer SB, McCants ML. *J Allergy Clin Immunol* 1987;80(2):133-139.
25. Laemmli UK. *Nature* 1970;227(259):680-685.
26. Demeulemester CG, Peltre, G., Laurente, M. *Electrophoresis* 1987(8):71-73.
27. Kyhse-Andersen J. *Electroblotting of multiple gels: J Biochem Biophys*
25 *Methods* 1984;10(3-4):203-209.
28. Leary JJ, Brigati DJ, Ward DC. *Proceedings of the National Academy of Sciences of the United States of America* 1983;80(13):4049-4049.
29. Rabjohn P, Helm EM, Stanley JS, et al. *J Clin Invest* 1999;103(4):535-542.
30. Stanley JS, King N, Burks AW, et al. *Arch Biochem Biophys*
30 1997;342(2):244-253.
31. Burks AW, Shin D, Cockrell G, Stanley JS, Helm RM, Bannon GA. *Eur J Biochem* 1997;245(2):334-339.
32. Pomes A, Melen E, Vailes LD, Retief JD, Arruda LK, Chapman MD. *JBiol Chem* 1998;273(46):30801-30807.
- 35 33. Beezhold DH, Hickey VL, Slater JE, Sussman GL. *J Allergy Clin Immunol* 1999;103(6):1166-1172.34. Muller U, Akdis CA, Fricker M, et al. *J Allergy Clin Immunol* 1998;101(6 Pt 1):747-754.
35. Leung PS, Chow WK, Duffey S, Kwan HS, Gershwin ME, Chu KH. *J Allergy Clin Immunol* 1996;98(5 Pt 1):954-961.
- 40 36. Pascual CY, Crespo JF, San Martin S, et al. *Allergy* 1997;52(5):514-520.

- 5 37. Reese G, Ayuso R, Carle T, Lehrer SB. International Archives of Allergy &
 Immunology 1999;11.8(24):300-301.
38. J.S. Stanley, N. King, A.W. Burks, S.K. Huang, H. Sampson, G. Cockrell,
 R.M. Helm, C.M. West, G.A. Bannon, Arch Biochem Biophys 342 (1997) 244.
39. A.W. Burks, N. King, G.A. Bannon, Int Arch Allergy Immunol 118 (1999)
10 313.
40. G.A. Bannon, D. Shin, S. Maleki, R. Kopper, A.W. Burks, Int Arch Allergy
 Immunol 118 (1999) 315.

- 5 What is claimed is:
1. A vaccine comprising at least one modified amino acid, of crustacea tropomyosin sequence; or a fragment thereof.
 2. The vaccine of claim 1 wherein the modified amino acid sequence reduces
10 binding between crustacea tropomyosin and an IgE antibody by at least 45% as determined by a standard IgE antibody test.
 3. The vaccine of claim 2 wherein the modified amino acid sequences includes
15 an epitope with at least one amino acid substitution that reduces the binding in the test.
 4. The vaccine of claim 1 wherein the modified amino acid substitutions include at least one of a non-polar aliphatic group; a polar, uncharged group; an aromatic group; a positively charged group; or a negatively charged group.
20
 5. A vaccine comprising crustacea tropomyosin or a fragment thereof.
 6. A method for desensitizing an individual allergic to crustacea tropomyosin, the method comprising administering to the individual a desensitization sufficient
25 amount of a vaccine comprising at least one modified amino acid of crustacea tropomyosin; or a vaccine comprising a modified fragment of crustacea tropomyosin.
 7. The method according to claim 6 wherein the administration is by at least
30 one of oral, suppository, parenteral, or subcutaneous administration.
 8. A vector comprising nucleic acid sequence encoding a modified crustacea tropomyosin, the modification comprising at least one amino acid substitution or deletion in an epitope capable of binding an IgE antibody, wherein the vector can
35 be selected from viruses, plasmids, bacterial, yeast.
 9. The vector of claim 8 comprising modified gene fragments of crustacea tropomyosin.

- 5 10. The vector of claim 8 comprising at least one nucleic acid substitution which reduces binding between crustacea tropomyosin and an IgE antibody by at least 45% as determined by a standard IgE antibody test.
- 10 11. The vector of claim 8 or 9 wherein the nucleic acid substitutions code for modified peptides that abolish or reduce between crustacea tropomyosin and an IgE antibody by at least 45% as determined by a standard IgE antibody test.
- 15 12. A peptide comprising amino acid sequence of the tropomyosin molecule of crustacea, or any fragment thereof.
13. A peptide according to claim 12, wherein the crustacean tropomyosin is shrimp tropomyosin.
- 20 14. A peptide according to claim 12, wherein the peptide is five or more amino acids long.
15. A peptide according to claim 12, wherein the peptide overlaps by at least two amino acids with the next consecutive peptide at the 3' end of the previous peptide.
- 25 16. A peptide according to claim 12, wherein said peptide evokes a positive skin reaction in a patient sensitive to ingestion of crustaceans.
- 30 17. A method for desensitizing an individual allergic to crustacea. the method comprising administering to the individual a desensitization sufficient amount of the peptide of claim 12.
- 35 18. A peptide according to claim 12, comprising at least one amino acid substitution which reduces binding between crustacea tropomyosin and an IgE antibody by at least about 45% as determined by a standard IgE antibody test.
- 40 19. An amino acid substitution according to claim 18, wherein the substitutions can be selected from any group of amino acids which are different to the amino acid group that is being substituted.

- 5 20. An amino acid substitution according to claim 19, wherein the substituting amino acid include at least one of a non-polar aliphatic group; a polar, uncharged group; an aromatic group; a positively charged group; or a negatively charged group.
- 10 21. A peptide according to claim 18, wherein said crustacea tropomyosin-specific IgE antibodies are shrimp tropomyosin-specific IgE antibodies and said crustacea tropomyosin is shrimp tropomyosin.
- 15 22. A method for treating an individual allergic to crustacea, said method comprising administering to the individual a sufficient amount of the peptide of claim 18, which reduces binding between crustacea tropomyosin and an IgE antibody by at least about 45% as determined by a standard IgE antibody test.
- 20 23. The method according to claim 22, which can be used to perform allergan specific therapy of patients allergic to other crustaceans wherein the administration is by at least one of oral, suppository, parenteral, or subcutaneous administration.
- 25 24. A method according to claim 23, which reduces binding between crustacea tropomyosin and an IgE antibody by at least about 45% as determined by a standard IgE antibody test.
- 25 25. The method according to claim 23, which can be used to perform allergan specific therapy of patients allergic to arthropods.
- 30 26. A method according to claim 25, which reduces binding between arthropod tropomyosin and an IgE antibody by at least about 45% as determined by a standard IgE antibody test.
- 35 27. A vector comprising the unmodified gene of crustacea tropomyosin; or a fragment thereof, wherein the vector can be selected from viruses, plasmids, bacterial, yeast.
28. A method for desensitizing an individual allergic to crustacea tropomyosin, the method comprising administering to the individual a desensitization sufficient

- 5 amount of a vector comprising the unmodified gene of crustacea tropomyosin; or a vector comprising an unmodified fragment of crustacea tropomyosin.
29. The method according to claim 28 wherein the administration is by at least one of oral, suppository, parenteral, or subcutaneous administration.
- 10 30. A peptide comprising amino acid sequences of the five major allergenic regions of tropomyosin, and fragments thereof, wherein said peptides evoke a positive skin reaction in a patient sensitive to ingestion of crustaceans.
- 15 31. A peptide according to claim 30, wherein substitution of at least one amino acids reduces binding between crustacea tropomyosin and an IgE antibody by at least about 45% as determined by a standard IgE antibody test.
- 20 32. An amino acid substitution according to claim 30, wherein the substitutions can be selected from any group of amino acids which are different to the amino acid group that is being substituted.
- 25 33. An amino acid substitution according to claim 32, wherein the substituting amino acid include at least one of a non-polar aliphatic group; a polar, uncharged group; an aromatic group; a positively charged group; or a negatively charged group.
- 30 34. An amino acid substitution according to claim 30, wherein the substitution is located anywhere in the allergenic epitope.
- 35 35. An amino acid substitution according to claim 30, wherein the substitution is located in the center of the allergenic epitope.
36. A method for desensitizing an individual allergic to crustacea tropomyosin, the method comprising administering to the individual a desensitization sufficient amount of the peptide according to claim 30.
- 40 37. The method according to claim 36 wherein the administration is by at least one of oral, suppository, parenteral, or subcutaneous administration.

- 5 38. The method of claim 36 wherein the individual is allergic to shrimp tropomyosin.
39. The method of claim 36 wherein the individual is allergic to other crustacea tropomyosin.
- 10 40. The method of claim 36 wherein the individual is allergic to arthropod tropomyosin.
41. Use of any of the peptides in any one of the above claims and
15 pharmaceutically acceptable derivatives and salts thereof for preparation of a medicament for the treatment of an inflammatory disorder.
42. Expression of any of the peptides in any one of the above claims, in
20 recombinant host selected from the group comprising bacteria, yeast, fungi, insect, crustacea, and mammalian cells.
43. A transgenic animal expressing a modified allergen which is less reactive
with IgE comprising at least one IgE binding site present in the allergen modified
by at least one amino acid change so that the site no longer binds IgE.
- 25 44. An antibody that specifically binds a modified tropomyosin molecule.

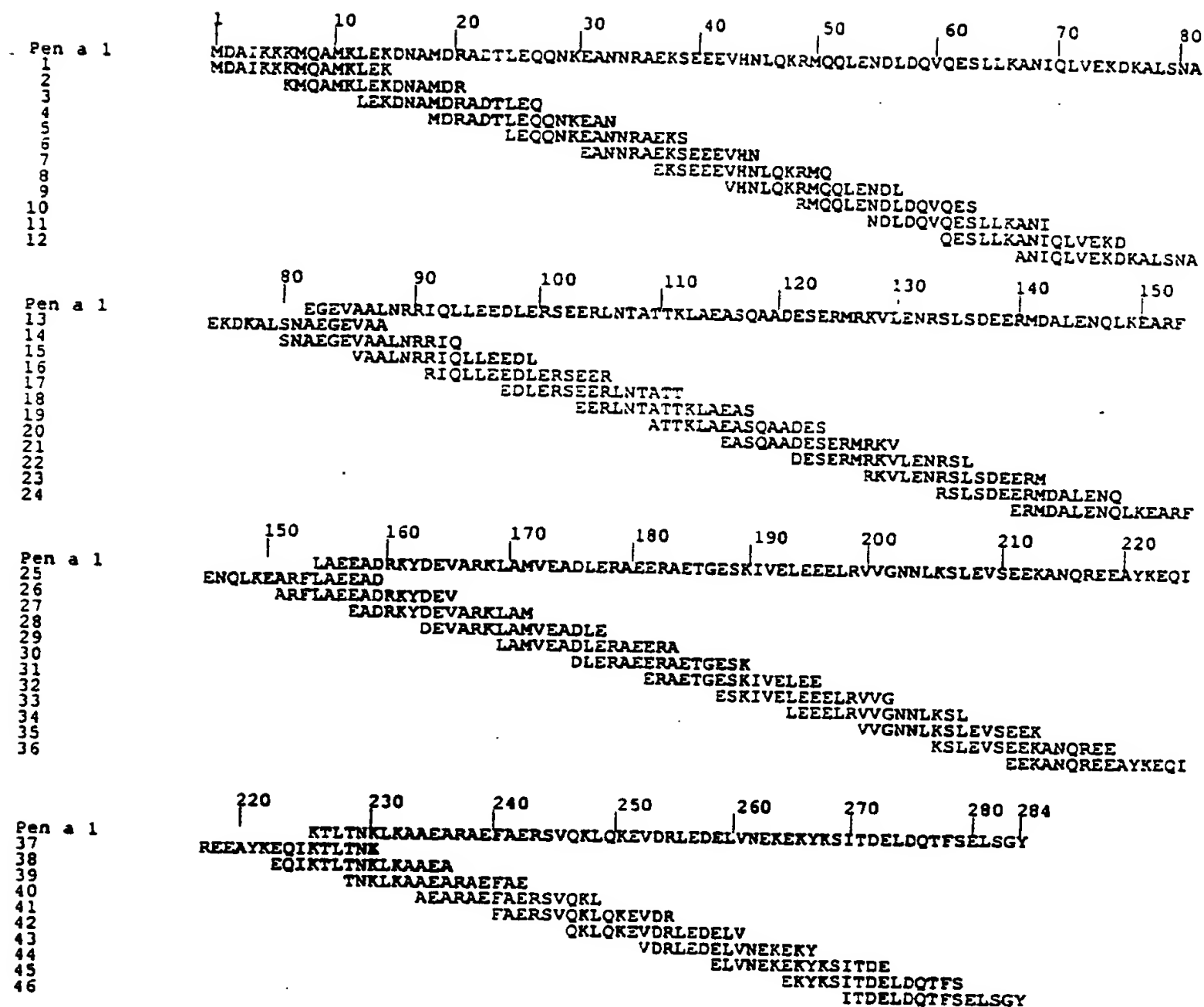
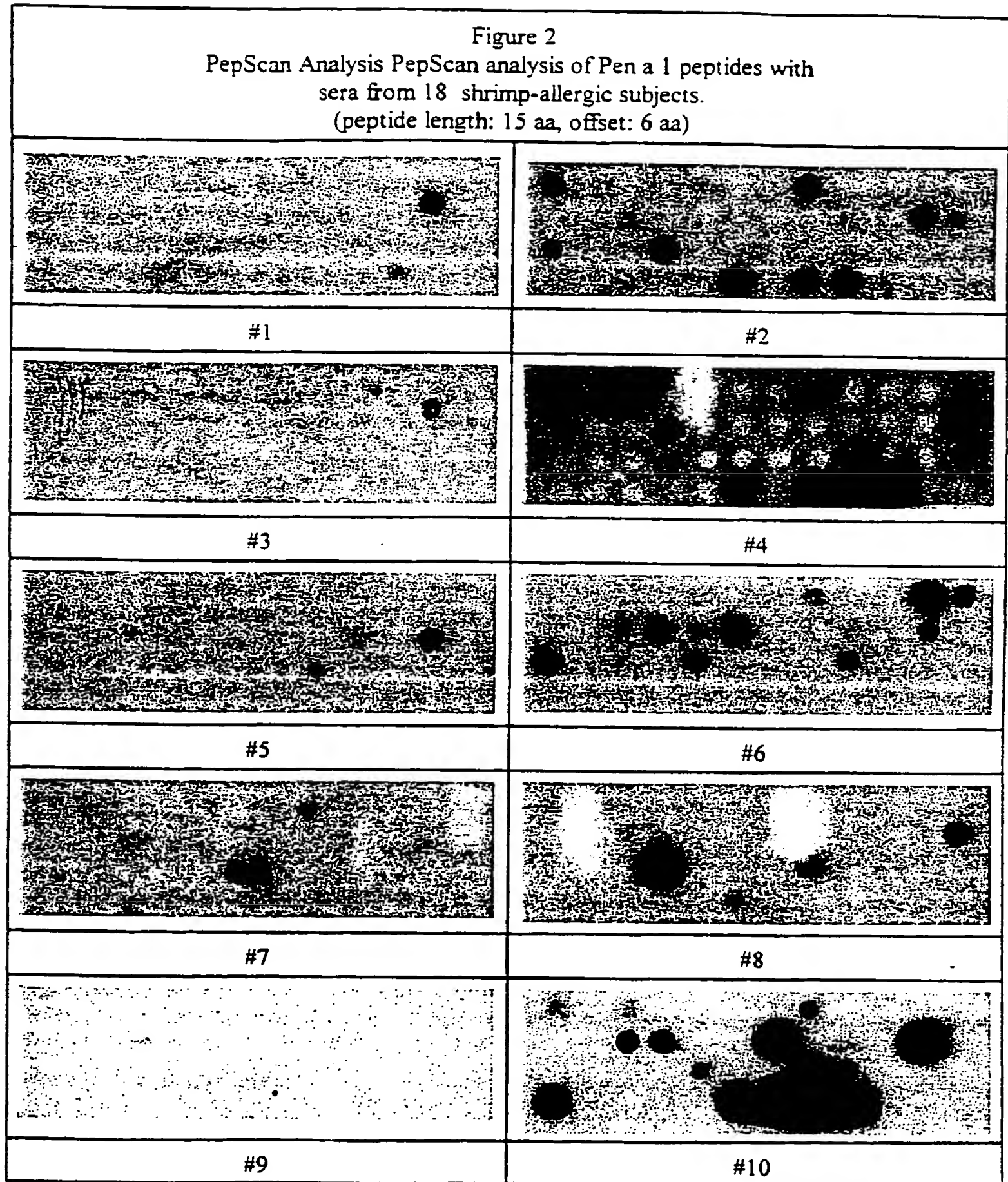


Figure 1: Forty-six, synthetic overlapping peptides spanning the entire sequence of Pen a 1 (length: 15 amino acid residues, offset: 6 amino acids)



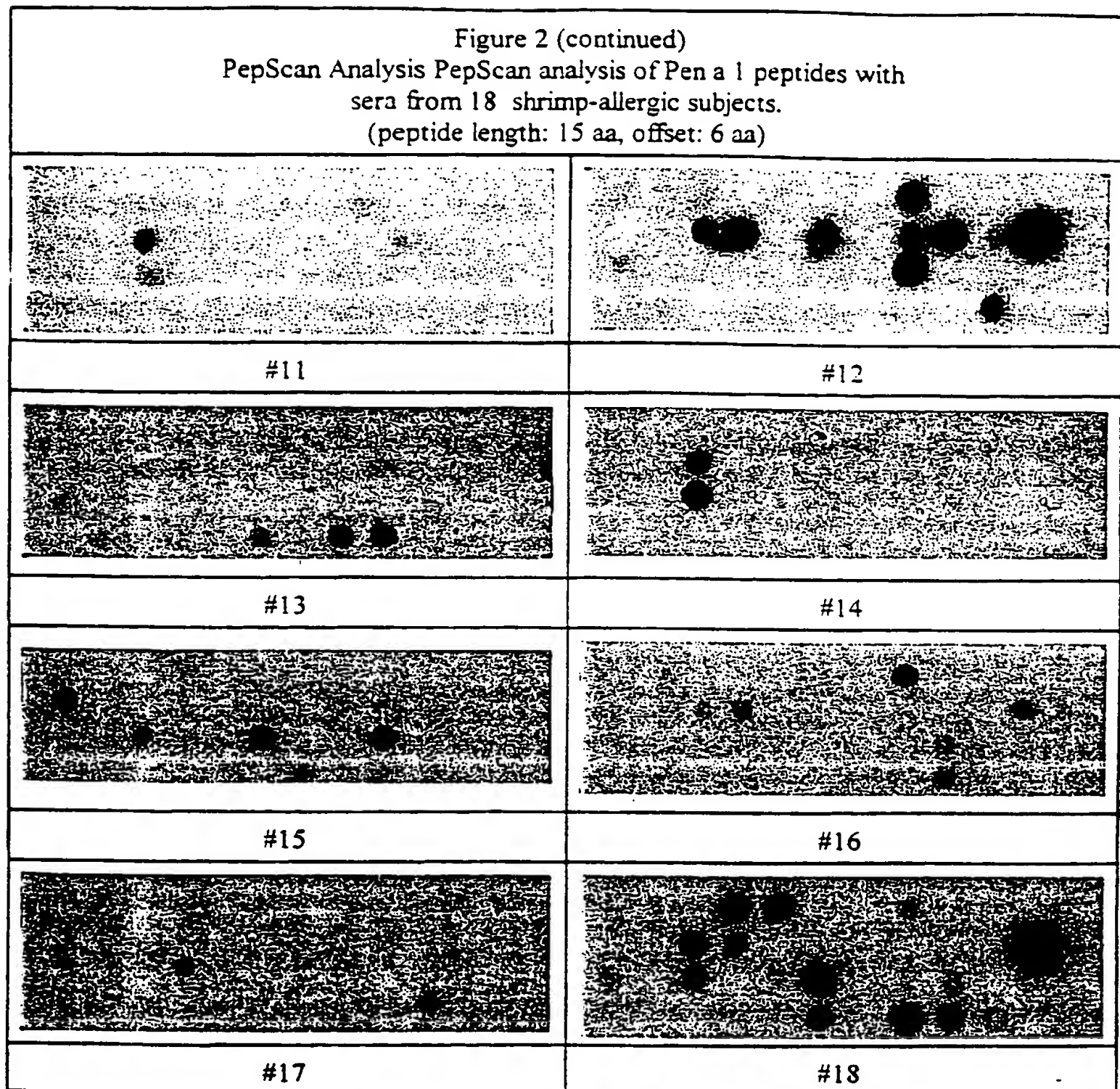


Figure 3.1:
Region 1: individually recognized epitopes
and sequence comparison with allergenic and non-allergenic tropomyosins

Pen a 1 peptide used for initial screening	position 43-57	37 40 50 60 63 EKSEEVHNLQKTHHCKLENDLDQVQES VHNLQKRMQOLEN	peptide 8
	Position		Subject
	43-55	VHNLQKRMQOLEN	2
	43-55	VHNLQKRMQOLEN	4
	43-55	VHNLQKRMQOLEN	12
	43-55	VHNLQKRMQOLEN	16
Met e 1		
Hom a 1		
Der p 10		..T...IRITH.K...V..E.....Q	
Der p 10		..A.....	
Der p 10		..A...ARS...KI..I.....TM.Q	
Der p 10	RA...KI..I..E.....Q	
Der p 10	RA...KI..I..E.....Q	
Der p 10		KQL..D.LVA...KLKGT..DE..KYS..	
Der p 10		KQL...QQG...KLKGT..DEVEKYS..	
Der p 10		KQL..D.LVS...KLKAT..DE..KYS.A	
Der p 10		KQL...QQA...KLKGT..DEVEKYS..	
Der p 10		KQDDALIQH...KLKGT..DE..KYS.A	
Der p 10		KQL..D.LLS...NLKGT..DE..KYS.A	

Crustacea: *Penaeus aztecus* (brown shrimp, Pen a 1), *Melapenaeus ensis* (greasy-back shrimp, Met e 1), *Homarus americanus* (Atlantic lobster, Hom a 1), *H. americanus* slow muscle tropomyosin (HomaTMs), *Panulirus stimpsoni* (spiny lobster, Pan s 1). Insecta: *Periplaneta americana* (American cockroach, Per a 7), Arachnida: *Dermatophagoides pteronyssinus*, *D. farinae* (house dust mites, Der p 10, Der f 10). Vertebrata: *Gallus Gallus* (chicken, alpha-tropomyosin GalgβTM; beta-tropomyosin GalgβTM), *Sus scrofa* (pig, SussβTM), *Salmo trutta* (Atlantic salmon, SaltTM), *Oryctolagus cuniculus* (rabbit, OrycβTM) (GenBank data).

Figure 3.2:
Region 2: individually recognized epitopes
and sequence comparison with allergenic and non-allergenic tropomyosins

Pen, & l peptides used for Initial screening	position	70	80	90	100	110	120	peptide 15 peptide 16 peptide 17
		QLVEKDKALSNAGEGEVAALNRRIQLLEEDLERSEERLNTATTKLAESQAA						
		VAALNRRIQLLEEDL						
		RIQLLEEDLERSEER EOLERSEERLNTATT						
Position		Subject					IgE-reactive peptides during Initial screening	
87-101		ALNRRIQLLEEDLER					6	15, 16, 17
87-101		ALNRRIQLLEEDLER					10	15, 16
87-101		ALNRRIQLLEEDLER					18	15, 16
91-101		RIQLLEEDLER					12	15, 16
<hr/>								
Met e 1							
Hom a 1		..E.....						
HomaTMS		K.E..E...Q.....						
Pan s 1		..E.....						
Per a 7		K.D.....Q...S.....						
Der p 10		K.E..E...QT...D.....I.....					A..A.....V	
GalgaTM		K.ELA..KATD..S...S.....V..E.D.AQ...A..LQ..E..EK..					KI..A..E...S.	
GalgbTM		K.ELA..KATD..S...S.....V..E.D.AQ...A..LQ..E..EK..					A..LQ..E..EK..	
SussaTM1		K.ELA..KATD..S...S.....V..E.D.AQ...A..LQ..E..EK..					A..LQ..E..EK..	
OrycaTM1		K.ELA..KATD..S...S.....V..E.D.AQ...A..LQ..E..EK..					A..LQ..E..EK..	
OrycβTM2		K.ELA..KATD..S...S.....V..E.D.AQ...A..LQ..E..EK..					A..LQ..E..EK..	
SaltTM1		K.ELA..KATD..S...S.....V..E.D.AQ...A..LQ..E..EK..					A..LQ..E..EK..	
SaltTM2		K.ELA..KATD..S...S.....V..E.D.AQ...A..LQ..E..EK..					A..LQ..E..EK..	

Crustacea: *Penaeus aztecus* (brown shrimp, Pen a 1), *Metapenaeus ensis* (greasy-back shrimp, Met e 1), *Homarus americanus* (Atlantic lobster, Hom a 1), *H. americanus* slow muscle tropomyosin (HomaTMS), *Panulirus stimpsoni* (spiny lobster, Pan s 1). Ins cta: *Peniplaneta americana* (American cockroach, Per a 7), Arachnida: *Dermatophagoides pteronyssinus*, *D. farinae* (house dust mites, Der p 10, D r f 10). Vertebrata: *Gallus Gallus* (chicken, alpha-tropomyosin GalgaTM; beta-tropomyosin GalgβTM), *Sus scrofa* (pig, SussβTM), *Salmo trutta* (Atlantic salmon, SaltTM), *Oryctolagus cuniculus* (rabbit, OrycβTM) (GenBank data).

Figure 3.3:
Region 3: Individually recognized epitopes
and sequence comparison with allergenic and non-allergenic tropomyosins

Pen a 1, Hom a 1, Pan a 1 peptides used for initial screening	position	125	130	140	150	160	Peptide 23	Peptide 24
	133-143							
	145-159	RMKVLNRSLSDEERMDALENQLKEARFLAEEDRK						
		RSLSDDEERMDALENQ						
		ERMDALENQLKEARF						
<hr/>								
Position							Subject	IgE-reactive peptides during initial screening
137-145		DEERMDALE					18	23
137-145		DEERMDALE					10	23
133-141		RSLSDDEERM					12	23
135-146		LSDEERMDALEN					1	23
134-141		SLSDEERM					2	23
136-143		SDEERMDA					5	23
144-151		MENORKEQ					6	23
139-153		ERMDALENQLKEARF					4	24
<hr/>								
Met a 1							
Hom a 1							
Hom a 1							
Pan a 1							
Per a 7							
Der p 10							
Der f 10							
Gal g 10							
Gal g 10							
Suss s 10							
Oryz s 10							
Oryz s 10							
Salt s 10							
Salt s 10							

Crustacea: *Penaeus aztecus* (brown shrimp, Pen a 1), *Melapenaeus ensis* (greasy-back shrimp, Met e 1), *Homarus americanus* (Atlantic lobster, Hom a 1), *H. americanus* slow muscle tropomyosin (Homa TMs), *Panulirus simpsoni* (lobster, Pan s 1), Insecta: *Periplaneta americana* (American cockroach, Per a 7), Arachnida: *Dermatophagoides pteronyssinus*, *D. fanniae* (house dust mites, Der p 10, Der f 10), Vertebrata: *Gallus Gallus* (chicken, alpha-tropomyosin Galg a TM; beta-tropomyosin Galg b TM), *Sus scrofa* (pig, Suss s TM), *Salmo trutta* (Atlantic salmon, Salt s TM), *Oryctolagus cuniculus* (rabbit, Oryc b TM) (GenBank data).

Figure 3.4:
Region 4: individually recognized epitopes
and sequence comparison with allergenic and non-allergenic tropomyosins

Pen a 1, Hom a 1, Pan s 1 peptides used for initial screening	180	190	200	209	Peptide 32
	AEERAETGESKIVELEEEELRVVGNNIKSLEV				
	ESKIVELEEEELRVVG				
IgE-reactive peptides during initial screening	Position		Subject		
	187-201	ESKIVELEEEELRVVG	#8	32	
	187-199	ESKIVELEEEELRV	#12	32	
	187-197	ESKIVELEEEEL	#10	32	
<hr/>					
Metel				
HomaTM1				
HomaTM5				
PanelF.....				
Pera7S.....				
Derp10				
Derf10				
GalgTMLS...CA...KT.T...A				
GalgβTM	S...VA...CGD...KI.T...A				
SuscatM1LS.G.CA...KT.T...A				
OrycatM1LS.G.CA...KT.T...A				
OrycβTM1	S...VA...CGD...KI.T...A				
SaltTM1	T...LA.G.CA...KN.S...A				
SaltTM2VS.L.CSD...KN.T...A				

Crustacea: *Penaeus aztecus* (brown shrimp, Pen a 1), *Metapenaeus ensis* (greasy-back shrimp, Met e 1), *Homonus americanus* (Atlantic lobster, Hom a 1), *H. americanus* slow muscle tropomyosin (HomaTMs), *Panulirus stimpsoni* (spiny lobster, Pan s 1). Insecta: *Periplaneta americana* (American cockroach, Per a 7), Arachnida: *Dermatophagoides pteronyssinus*, *D. farinæ* (house dust mites, Der p 10, Der f 10). Vertebrata: *Gallus Gallus* (chicken, alpha-tropomyosin GalgαTM; beta-tropomyosin GalgβTM), *Sus scrofa* (pig, SussβTM), *Salmo trutta* (Atlantic salmon, SaltTM), *Oryzolagus cuniculus* (rabbit, OrycβTM) (GenBank data).

Figure 3.5:
Region 5: individually recognized epitopes
and sequence comparison with allergenic and non-allergenic tropomyosins

[illegible]

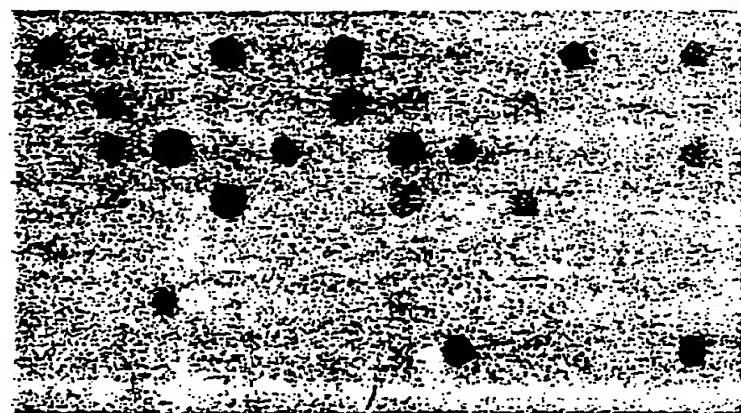
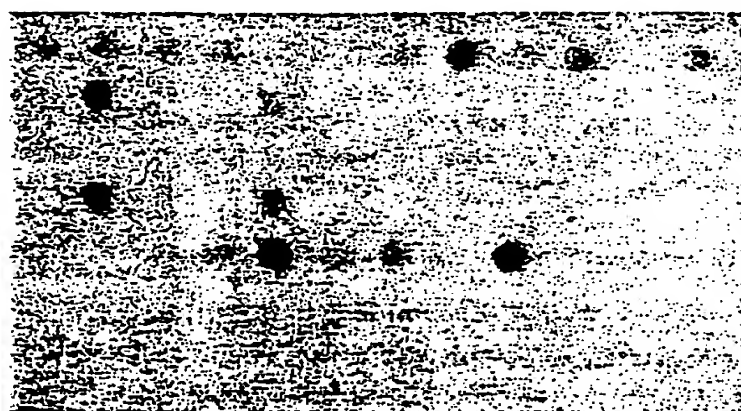
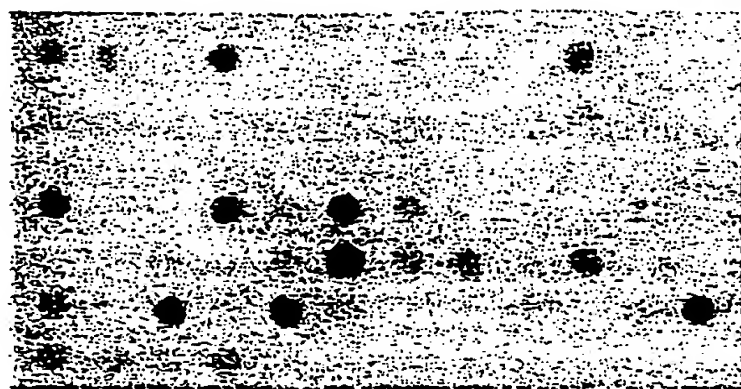
Met e l
Homa 1
HomaTMs
Pan e l
Ver b 7
Ver p 10
Ver f 10
GalgrM
GalgrM
SussotM1
OrycotM
OrycBTM2
SalcM1
SalcM2

Crustacea: *Penaeus aztecus* (brown shrimp, Pen a 1), *Penaeus aztecus* (greasy-back shrimp, Met e 1), *Homarus americanus* (Atlantic lobster, Hom a 1), *H. americanus* slow muscle tropomyosin (HomaTMs), *Panulirus stimpsoni* (spiny lobster, Pan s 1). Insecta: *P. niplaneta americana* (American cockroach, Per a 7), Arachnida: *Dermatophagoides pteronyssinus*, *D. farinae* (house dust mites, Der p 10, Der f 10). Vertebrata: *Gallus Gallus* (chicken, alpha-tropomyosin GalgαTM; beta-tropomyosin GalgβTM), *Sus scrofa* (pig, SussβTM), *Salmo trutta* (Atlantic salmon, SaliTM), *Oryctolagus cuniculus* (rabbit, OrycβTM) (GenBank data).

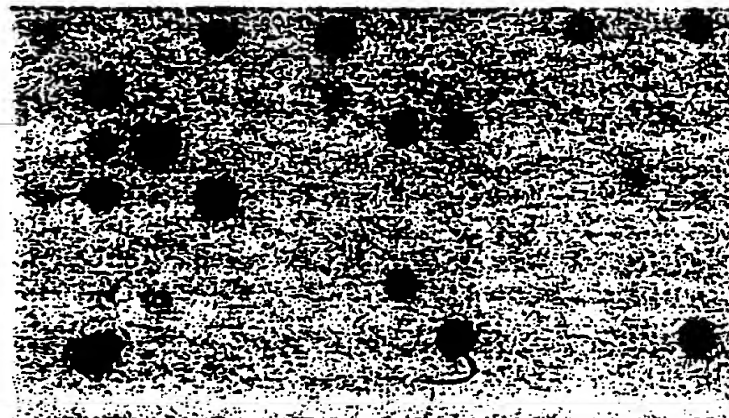
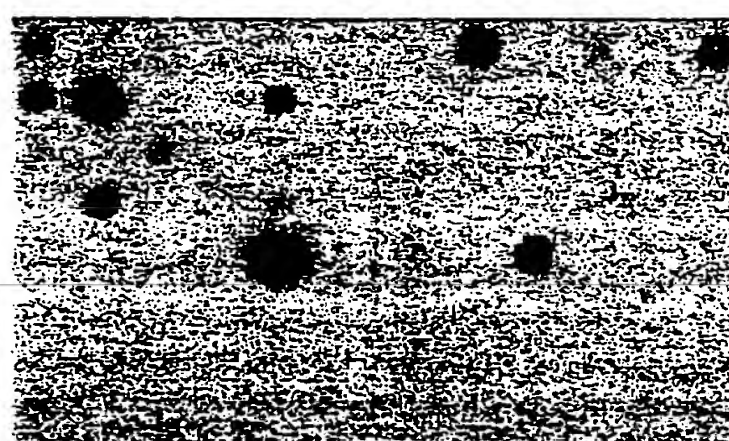
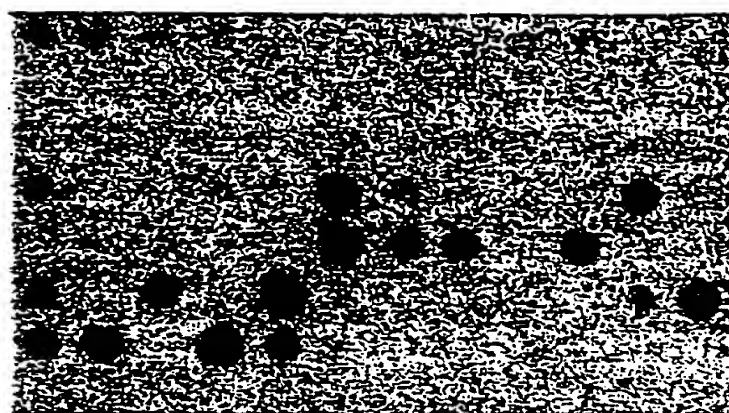
Pen a 1	ALSNAEGE	
# 2	K.....	1 substitution
# 3	.A.....	
# 4	..T.....	
# 5	...D.....	
# 6S.	
# 7	KA.....	2 substitutions
# 8	K.T.....	
# 9	K..D.....	
# 10	K.....S.	
# 11	.AT.....	
# 12	.A.D.....	3 substitutions
# 13	.A....S.	
# 14	..TD.....	
# 15	..T...S.	
# 16	...D..S.	
# 17	KAT.....	4 substitutions
# 19	KA.D.....	
# 19	KA....S.	
# 20	K.TD.....	
# 21	K.T...S.	
# 22	K..D..S.	5 substitutions
# 23	.ATD.....	
# 24	.AT...S.	
# 25	.A.D..S.	
# 26	..TD..S.	
# 27	KATD.....	
# 28	KAT...S.	
# 29	KA.D..S.	
# 30	K.TD..S.	
# 31	.ATD..S.	
chicken Tm	KATD..S.	

Figure 4: Peptides, resulting from combinatorial substitutions and transforming a Pen a 1 peptide into the homologous chicken tropomyosin (Tm) sequence.

Figure 5.1
Combinatorial Substitutions
Epitopes of region 1



#12



#4




Figure 5.2 Combinatorial Substitutions Region 2	
	
#6	#10
not done	
#18	# 12

Figure 5.3
Combinatorial Substitutions
Epitopes of region 3

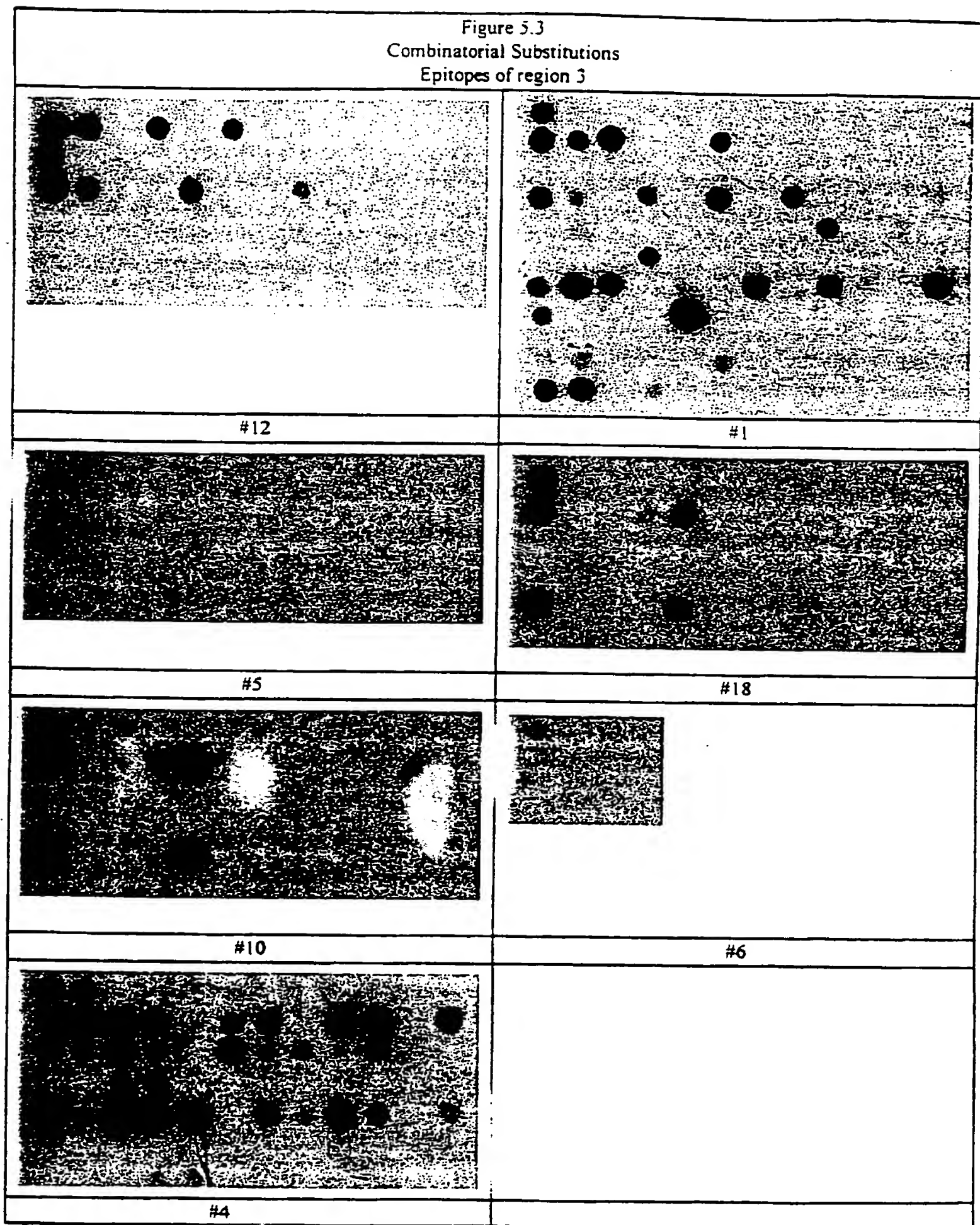
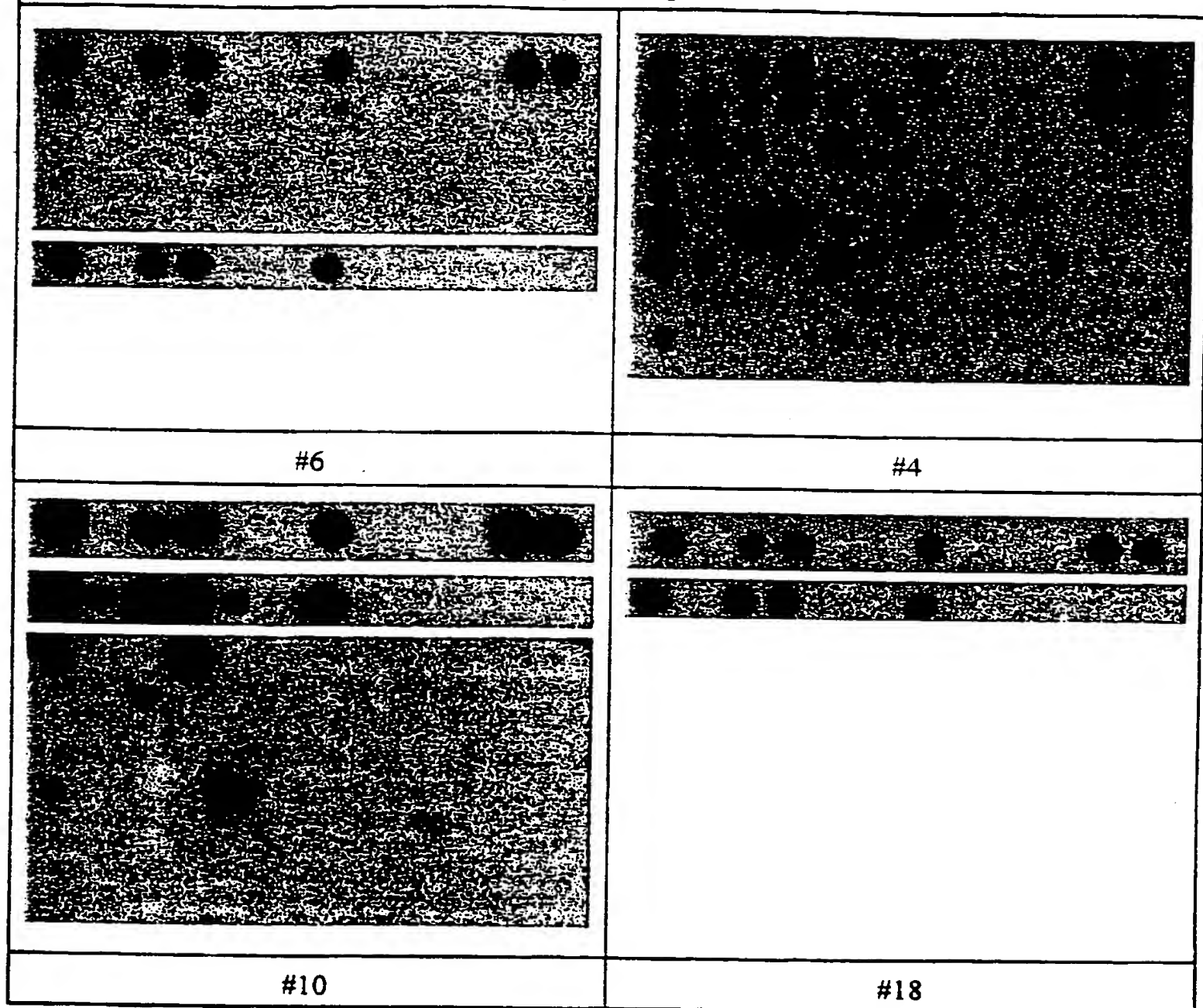


Figure 5.4
Combinatorial Substitutions
Epitopes of region 5



14/63

Figure 6

Critical Amino Acid Positions within Epitopes of Region 1

Position	37	40	50	60	63	critical position	mutated into amino acid	Subject
43-55	ENSEEEVNI	QKKH	QI	ENLD	QVCS	no	--	4
43-55	VHN	LQKR	QI	EN		no	--	12

Critical Amino Acid Positions within Epitopes of Region 2

Position	70	80	90	100	110	120	critical position	mutated into amino acid	Subject
87-101	QV	E	K	A	S	N	95	V, F	6
87-101	AL	N	R	I	Q	E	98	E	6
87-101	AL	N	R	I	Q	E	100	D	6
87-101	AL	N	R	I	Q	E	95	V, F	10
91-101	R	I	Q	E	D	L	95	F	12

Critical Amino Acid Positions within Epitopes of Region 3

Position	125	130	140	150	160	critical position	mutated into amino acid	Subject
137-145	R	H	K	V	L	140	K	18
137-145	U	E	N	M	A	147	E	18
137-145	D	E	E	M	A	149	K	10
137-145	D	E	E	M	A	142	E	10
133-141	R	S	S	D	E	135	Q, M, S	12
133-141	R	S	S	D	E	136	K	12
135-146	L	S	D	E	M	136	K	1
135-146	L	S	D	E	M	142	E	1
136-143	S	D	E	M	A	136	K	5
136-143	S	D	E	M	A	140	K	5
144-151	E	R	M	A	S	142	E	5
139-153	E	R	M	A	S	144	Q	6

Critical Amino Acid Positions within Epitopes of Region 5

Position	239	250	260	270	280	284	critical position	mutated into amino acid	Subject
249-260	A	S	E	V	N	E	255	D	2
249-261	L	S	D	E	M	A	250	E	10
249-261	L	S	D	E	M	A	252	S	10
249-261	L	S	D	E	M	A	255	D	10
249-261	L	S	D	E	M	A	260	V	10
251-259	K	Y	N	I	T	D	255	D	4
266-273	K	Y	N	I	T	D	269	F	2
266-273	K	Y	N	I	T	D	269	F	10
266-273	K	Y	N	I	T	D	269	F	4
273-281	E	R	M	A	S		269	F	18
273-281	E	R	M	A	S		277	A	4
273-281	E	R	M	A	S		278	L	4
273-281	E	R	M	A	S		280	Q	4
273-281	E	R	M	A	S		281	N, I	4

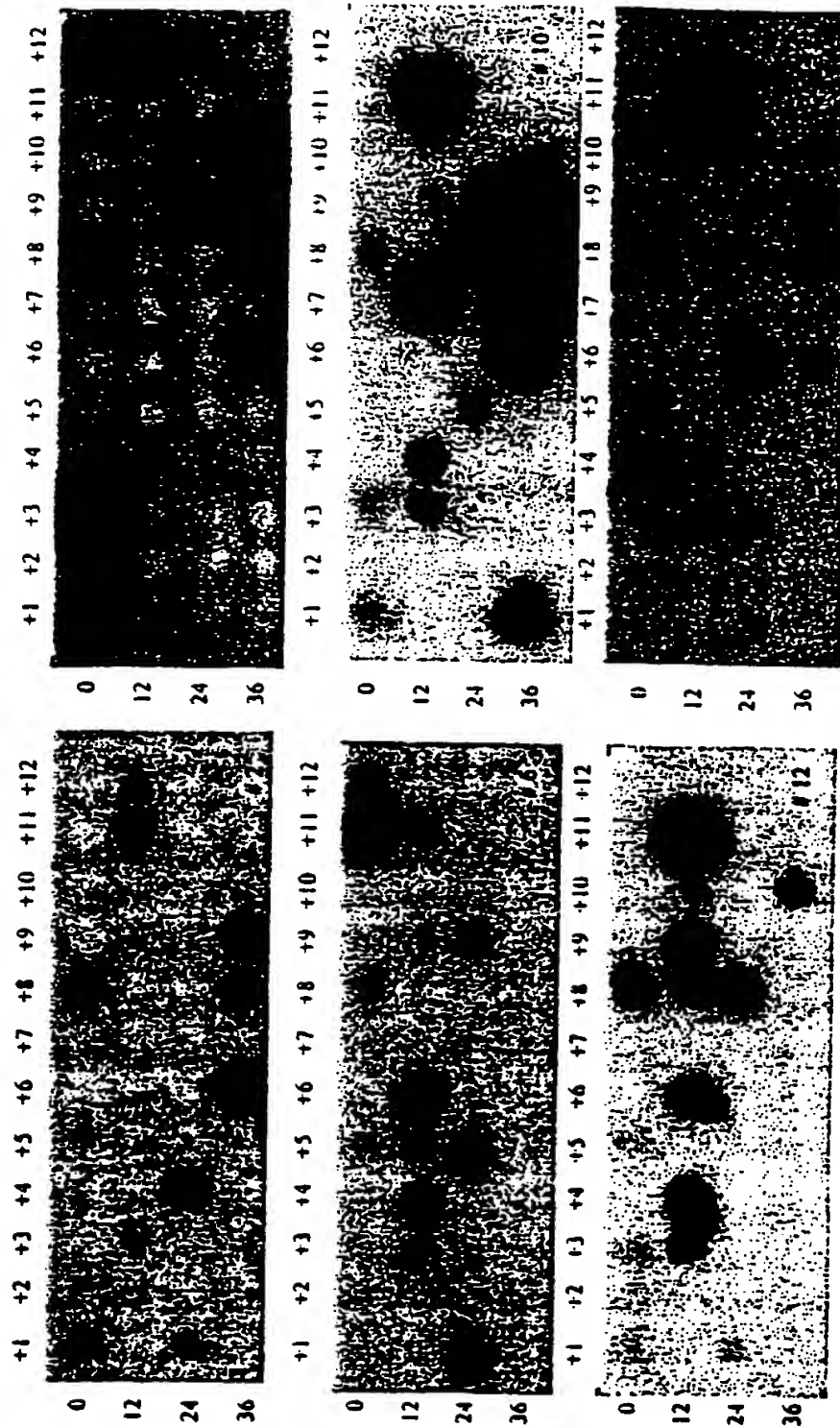


Figure 7

figure 7

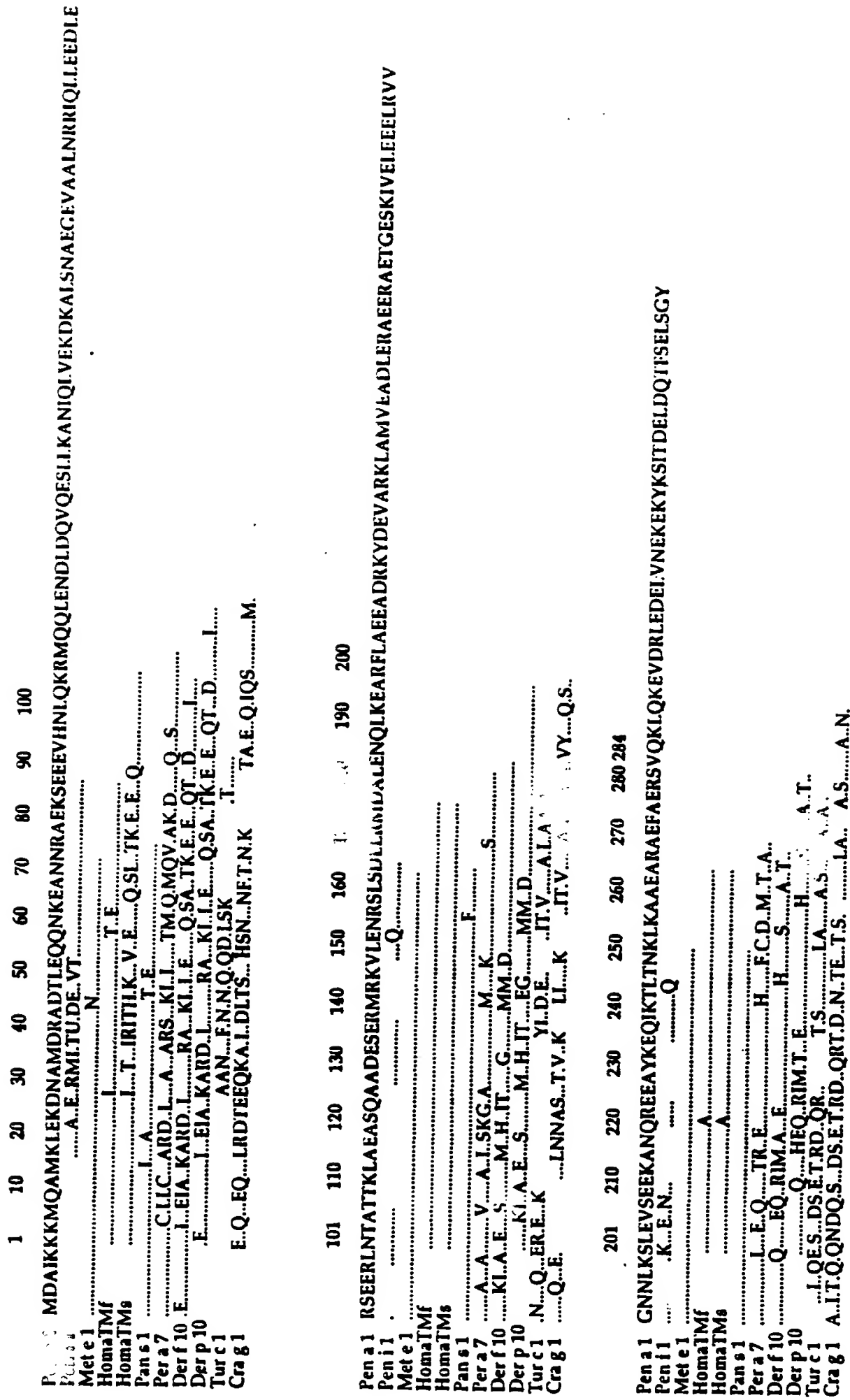


Figure 8: Comparison of identified Pen a 1 IgE-binding epitopes with homologous sequences of arthropod tropomyosins from the shrimp *Metapenaeus ensis* (Met e 1), American lobster *Homarus americanus* slow and fast isoforms (Homa 1ms, Homa 1mf), spiny lobster *Panulirus stimpsoni* (Pan s 1), house dust mites *Dermatophagoides pteronissinus* and *D. farinae* (Der p 10, Der f 10) and cockroach *Periplaneta americana* (Pera 7), snail *Turbo cornutus*, and oyster *Crassostrea gigas*.

Figure 8

IgE-binding, recombinant peptides		non-IgE-binding synthetic peptides	
157-169	EADRKYEVARKL	157 - 171	EADRKYEVARKLAM
167-179	RKLAMVEADLERA	163 - 177	DEVARKLAMVEADLE
		169 - 183	LAMVEADLERAEEA

9

Figure 1:

Sequence comparison of IgE-binding, recombinant peptides and non-IgE-binding synthetic peptides: Identical sequences are shaded.

Figure 1

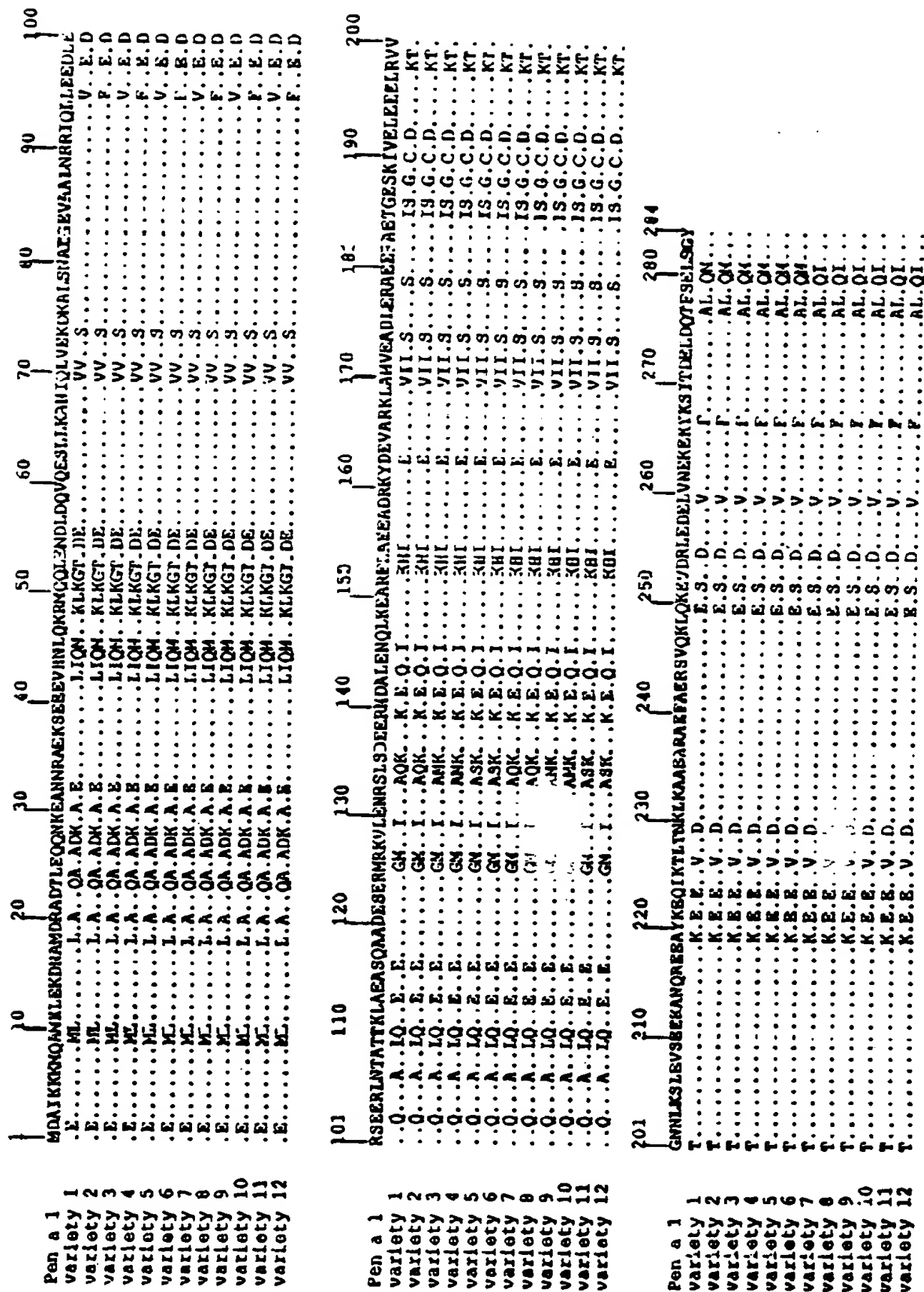


Figure 8: This figure shows 12 Pen a 1 varieties that contain substitutions in 78 positions and will reduce or abolish the IgE antibody reactivity of the Pen a 1 molecule (see Table 4). The varieties represent sequences that contain the maximal number of substitutions; not all substitutions may be necessary since the effect of multiple substitutions is not always known.

Figure 10

Table 1: Summary of PepScan analysis of IgE antibody reactivities of 18 shrimp-allergic subjects to 46 overlapping peptides spanning the entire length of Pen a 1.

Table 2

IgE-reactive, peptides identified by PepScan of Pen a 1		IgE-reactive, peptides identified by SPOTSIZE of Pen a 1 region 1		IgE-reactive, peptides identified by SPOTSIZE of Pen a 1 region 2	
Pen a 1 1-15	NDAIKKKKQAKLEK	Pen a 1 43-57	VHNLQKRMOOLENDL	Pen a 1 85-99	VVALNRRIQLEEDL
Pen a 1 17-21	KKQAKKLEKNANDR	Pen a 1 43-56	VHNLQKRMOOLEND	Pen a 1 88-100	AALNRRIQLEEDLE
Pen a 1 13-27	LEKNADHRAADTLEQ	Pen a 1 43-55	VHNLQKRMOOLEN	Pen a 1 07-101	AINRRIQLEEDLER
Pen a 1 19-33	MDRADTLEQKKEAN	Pen a 1 43-54	VHNLQKRMOOLE	Pen a 1 88-101	INRRIQLEEDLER
Pen a 1 25-38	LEQKKEKNRAEKS	Pen a 1 44-56	HNQLQKRMOOLEND	Pen a 1 88-102	IJRRIQLEEDLERS
Pen a 1 43-57	VHNLQKRMOOLENDL	Pen a 1 45-57	NLQKRMOOLENDL	Pen a 1 89-101	NRRIQLEEDLER
Pen a 1 61-75	QESLLKANTQLVEKD	Pen a 1 45-56	NLQKRMOOLEND	Pen a 1 89-102	NRRIQLEEDLERS
Pen a 1 67-81	ANTQLVEKDKALSNA	Pen a 1 46-57	LQKRMOOLENDL	Pen a 1 89-103	NRRIQLEEDLERSE
Pen a 1 73-87	EKKKALSNAEGEVA			Pen a 1 90-104	RRIQLEEDLERSEE
Pen a 1 85-99	VVALNRRIQLEEDL			Pen a 1 90-103	RRIQLEEDLERSE
Pen a 1 91-105	RIQLEEDLERSEER			Pen a 1 90-102	RRIQLEEDLERS
Pen a 1 97-111	EDLERSEERLNTATT			Pen a 1 90-101	RRIQLEEDLER
Pen a 1 103-117	EERLNTATTKLAEAS			Pen a 1 91-101	RIQLEEDLER
Pen a 1 109-123	ATTKLAEASQAADES			Pen a 1 91-105	RIQLEEDLERSEER
Pen a 1 115-128	EASQAADESEKRRKV			Pen a 1 91-102	RIQLEEDLERS
Pen a 1 121-135	DESEKRRKVLENRSL			Pen a 1 92-101	IQLEEDLER
Pen a 1 127-141	RKVLHRSLSDEERH			Pen a 1 82-102	IQLEEDLERS
Pen a 1 133-147	RSLSDEERHDALENQ			Pen a 1 88-107	DLERSEERLN
Pen a 1 139-153	ERMDALENQLKEARF			Pen a 1 89-107	LERSEERLN
Pen a 1 145-159	ENQLKEARFLAEAD			Pen a 1 89-109	LERSEERLNTA
Pen a 1 163-177	DEVARKLAHVEADLE			Pen a 1 103-113	EERLNTATTKL
Pen a 1 169-183	LAVVEADLERAEERA			Pen a 1 104-113	ERLNTATTTKL
Pen a 1 175-189	ULLRAERNAETGRSK			Pen a 1 105-113	RLNTATTTKL
Pen a 1 187-201	ESKIVELEERELRVVC			Pen a 1 105-114	RLNTATTTKLA
Pen a 1 183-207	LEBELRVVGNLAKSL				
Pen a 1 211-225	EERKARQREAYKDOI				
Pen a 1 217-231	RECAKKEQIKTLTK				
Pen a 1 223-237	EQIKTLTKLKAADA				
Pen a 1 247-261	QKLOKEVDRIEDELV				
Pen a 1 253-267	VUNLEDELVNEKEY				
Pen a 1 259-273	ELVNEKEYKSITDE				
Pen a 1 265-278	ERYKSITDELDTQTS				
Pen a 1 270-284	ITDELDTQTSLSGY				

Table 2 (continued)					
IgE-reactive, peptides identified by SPOTSIZE of Pen a 1 region 3		IgE-reactive, peptides identified by SPOTSIZE of Pen a 1 region 4		IgE-reactive, peptides identified by SPOTSIZE of Pen a 1 region 5	
Pen a 1 126-133	MRKVLNRR	Pen a 1 187-198	ESKIVELEELR	Pen a 1 187-198	ESKIVELEELR
Pen a 1 127-133	RKVLNRR	Pen a 1 187-199	ESKIVELEELRV	Pen a 1 187-199	ESKIVELEELRV
Pen a 1 127-135	RKVLNRRSL	Pen a 1 187-200	ESKIVELEELRVV	Pen a 1 187-200	ESKIVELEELRVV
Pen a 1 132-139	NRSLSDDE	Pen a 1 187-201	ESKIVELEELRVVG	Pen a 1 187-201	ESKIVELEELRVVG
Pen a 1 133-139	RSLSDDE	Pen a 1 188-201	SKIVELEELRVVG	Pen a 1 188-201	SKIVELEELRVVG
Pen a 1 133-141	RSLSDDERM	Pen a 1 188-199	KIVELEELRV	Pen a 1 188-199	KIVELEELRV
Pen a 1 133-146	RSLSDDERMDALEN	Pen a 1 189-200	KIVELEELRVV	Pen a 1 189-200	KIVELEELRVV
Pen a 1 133-147	RSLSDDERMDALENQ	Pen a 1 189-201	KIVELEELRVVG	Pen a 1 189-201	KIVELEELRVVG
Pen a 1 134-139	SLSDEE	Pen a 1 190-201	IVELEELRVVG	Pen a 1 190-201	IVELEELRVVG
Pen a 1 134-144	SLSDEERMDAL	Pen a 1 191-200	VELEELRVV	Pen a 1 191-200	VELEELRVV
Pen a 1 134-146	SLSDEERMDALEN	Pen a 1 191-201	VELEELRVVG	Pen a 1 191-201	VELEELRVVG
Pen a 1 134-147	SLSDEERMDALENQ	Pen a 1 193-201	LEELRVVG	Pen a 1 193-201	LEELRVVG
Pen a 1 135-143	LSDEERMDA	Pen a 1 193-202	LEELRVVGN	Pen a 1 193-202	LEELRVVGN
Pen a 1 135-144	LSDEERMDAL	Pen a 1 195-202	EELRVVGN	Pen a 1 195-202	EELRVVGN
Pen a 1 135-145	LSDEERMDALE	Pen a 1 195-203	EELRVVGN	Pen a 1 195-203	EELRVVGN
Pen a 1 135-146	LSDEERMDALEN	Pen a 1 195-206	EELRVVGNILKS	Pen a 1 195-206	EELRVVGNILKS
Pen a 1 135-147	LSDEERMDALENQ	Pen a 1 196-207	EELRVVGNILKSL	Pen a 1 196-207	EELRVVGNILKSL
Pen a 1 136-143	SDEERMDA	Pen a 1 197-207	LRVVGNILKSL	Pen a 1 197-207	LRVVGNILKSL
Pen a 1 139-153	ERMDALENQLKEARF	Pen a 1 199-205	VVGNILK	Pen a 1 199-205	VVGNILK
Pen a 1 140-153	RMDALENQLKEARF				
Pen a 1 141-149	MDALENQLK				
Pen a 1 141-153	MDALENQLKEARF				
Pen a 1 144-149	LENQLK				
Pen a 1 144-151	LENQLKEA				

Table 3.1 Combinatorial Substitutions Region 1				
	Spot	peptide	#12	#4
Hom a TMs*	1	1		
	2	2	I.....	I.....
	3	3	.R.....	.R.....
	4	4		.I.....
	5	5	...T.....	...T.....
	6	6H.....H.....
	7	7K.....K.....
	8	8V.....V.....
	9	9	IR.....	IR.....
	10	10		I.I.....
	11	11	I..T.....	I..T.....
	12	12	I..H.....	I..H.....
	13	13	I....K.....	I....K.....
	14	14	I.....V.....	I.....V.....
	15	15	.RI.....	.RI.....
	16	16	.R.T.....	.R.T.....
	17	17	.R..H.....	.R..H.....
	18	18	.R...K.....	.R...K.....
	19	19	.R.....V.....	.R.....V.....
	20	20	..IT.....	..IT.....
	21	21	..I.H.....	..I.H.....
	22	22	..I..K.....	..I..K.....
	23	23	..I.....V.....	..I.....V.....
	24	24	...TH.....	...TH.....
	25	25	...T.K.....	...T.K.....
	26	26	...T.....V.....	...T.....V.....
	27	27	...H.K.....	...H.K.....
	28	28	...H.....V.....	...H.....V.....
	29	29K..V.....K..V.....
	30	30	IRITH.K..V.....	IRITH.K..V.....
Per a 7*	37	31		
	38	32	A.....	A.....
	39	33	.R.....	.R.....
	40	34		.S.....
	41	35K.....K.....
	42	36		
	43	37I..I..
	44	38	AR.....	AR.....
	45	39	A.S.....	A.S.....
	46	40	A....K.....	A....K.....
	47	41	A.....I..	
	48	42	A.....I..	A.....I..
	49	43	.RS.....	.RS.....
	50	44	.R...K.....	.R...K.....
	51	45	.R....I.....	.R....I.....
	52	46	.R.....I..	.R.....I..
	53	47	..S..K.....	..S..K.....
	54	48		
	55	49	..S.....I..	
	56	50KI.....	
	57	51K..I..K..I..
	58	52I..I..	
	59	53	ARS...KI..I..	ARS...KI..I..

Table 3.1
Combinatorial Substitutions
Region 1

	Spot	peptide	#12	#4
Hom a TMs*	1	1	VENLOKRMCOOLEN	VENLOKRMCOOLEN
	2	2	I.....	I.....
	3	3	R.....	R.....
	4	4		I.....
	5	5	T.....	T.....
	6	6	H.....	H.....
	7	7	K.....	K.....
	8	8	V.....	V.....
	9	9	IR.....	IR.....
	10	10		I.I.....
	11	11	I.T.....	I.T.....
	12	12	I.H.....	I.H.....
	13	13	I.K.....	I.K.....
	14	14	I.V.....	I.V.....
	15	15	RI.....	RI.....
	16	16	RT.....	RT.....
	17	17	R.E.....	R.E.....
	18	18	R.K.....	R.K.....
	19	19	R.V.....	R.V.....
	20	20	IT.....	IT.....
	21	21	I.H.....	I.H.....
	22	22	I.K.....	I.K.....
	23	23	I.V.....	I.V.....
	24	24	TH.....	TH.....
	25	25	T.K.....	T.K.....
	26	26	T.V.....	T.V.....
	27	27	E.K.....	E.K.....
	28	28	H.V.....	H.V.....
	29	29	K.V.....	K.V.....
	30	30	IRITH.K.V.	IRITH.K.V.
Per a 7*	37	31	VENLOKRMCOOLEN	VENLOKRMCOOLEN
	38	32	A.....	A.....
	39	33	R.....	R.....
	40	34		S.....
	41	35	K.....	K.....
	42	36		
	43	37	I.....	I.....
	44	38	AR.....	AR.....
	45	39	A.S.....	A.S.....
	46	40	A.K.....	A.K.....
	47	41	A.I.....	A.I.....
	48	42	A.I.....	A.I.....
	49	43	RS.....	RS.....
	50	44	R.K.....	R.K.....
	51	45	R.I.....	R.I.....
	52	46	R.I.....	R.I.....
	53	47	S.K.....	S.K.....
	54	48		
	55	49	S.I.....	S.I.....
	56	50	KI.....	KI.....
	57	51	K.I.....	K.I.....
	58	52	I.I.....	I.I.....
	59	53	ARS.KI.I.	ARS.KI.I.

Table 3.1 Combinatorial Substitutions Region 1 (continued)				
	Spot	peptide	#12	#4
Der p 10*	61	54I..I..
	62	55	RA.....	RA.....
	63	56	R...K.....	R...K.....
	64	57	R...I.....	R...I.....
	65	58	R.....I..	R.....I..
	66	59K.....K.....
	67	60K.....K.....
	68	61I..I..
	69	62	RA.....	RA.....
	70	63	R...K.....	R...K.....
	71	64	R...I.....	R...I.....
	72	65	R.....I..	R.....I..
	73	66K.....K.....
	74	67K.....K.....
	75	68I..I..
	76	69I..I..
	77	70	RA...KI...I..	RA...KI...I..

Table 3.1
Combinatorial Substitutions
Region 1 (continued)

	Spot	peptide	#12	#4
chicken α -TM*	97	71	LVENLQRMQOLEN	LVENLQRMQOLEN
	98	72	L.....	L.....
	99	73	.V.....	.V.....
	100	74K.....K.....
	101	75K.....K.....
	102	76K.....K.....
	103	77K.....K.....
	104	78G.....G.....
	105	79T.....T.....
	106	80D.....D.....
	107	81	LV.....	LV.....
	108	82K.....K.....
	109	83	L.....K.....	L.....K.....
	110	84	L.....L.....	L.....L.....
	111	85	L.....K.....	L.....K.....
	112	86	L.....G.....	L.....G.....
	113	87	L.....T.....	L.....T.....
	114	88D.....D.....
	115	89	.VA.....	.VA.....
	116	90	.V...K.....	.V...K.....
	117	91	.V...L.....	.V...L.....
	118	92	.V...K.....	.V...K.....
	119	93	.V...G.....	.V...G.....
	120	94	.V...T.....	.V...T.....
	121	95	.V...D.....	.V...D.....
	122	96K.....K.....
	123	97G.....G.....
	124	98	..A....K....	..A....K....
	125	99G.....G.....
	126	100	..A....T....	..A....T....
	127	101D.....D.....
	128	102KL.....KL.....
	129	103K.K.....K.K.....
	130	104K.G.....K.G.....
	131	105K.T.....K.T.....
	132	106K...DK...D
	133	107LK.....LK.....
	134	108L.G.....L.G.....
	135	109L.T.....L.T.....
	136	110D.....D.....
	137	111KG.....KG.....
	138	112K.T.....K.T.....
	139	113K...DK...D
	140	114GT.....GT.....
	141	115G...DG...D
	142	116T.DT.D
	143	117	LVA...KLKGT.D	LVA...KLKGT.D
rabbit α -TM*	144	118	..S.....G....	..S.....G....
	145	119	LVS...KLKGT.D	LVS...KLKGT.D

Table 3.1
Combinatorial Substitutions
Region 1 (continued)

	Spot	peptide	#12	#4
chicken β -TM*	146	120	Q.....	Q.....
	157	121	.Q.....	.Q.....
	158	122	..G.....	..G.....
	159	123	QQ.....	QQ.....
	160	124	Q.G.....	Q.G.....
	161	125	Q....K....	Q....K....
	162	126	Q....L....	Q....L....
	163	127	Q....K....	Q....K....
	164	129	Q....G....	Q....G....
	165	129	Q....T....	Q....T....
	166	130	Q.....D	Q.....D
	167	131	.QG.....	.QG.....
	168	132	.Q....K....	.Q....K....
	169	133	.Q....L....	.Q....L....
	170	134	.Q....K....	.Q....K....
	171	135	.Q....G....	.Q....G....
	172	136	.Q....T....	.Q....T....
	173	137	.Q.....D	.Q.....D
	174	138	.G....K....	.G....K....
	175	139	.G....L....	.G....L....
	176	140	.G....K....	.G....K....
	177	141	.G....G....	.G....G....
	178	142	.G....T....	.G....T....
	179	143	.G....D....	.G....D....
	180	144	QQG...KLKGT.D	QQG...KLKGT.D
swineTM*	181	145	..S.....	..S.....
	193	146A...A...
	194	147	L.S.....	L.S.....
	195	148	L.....A...	L.....A...
	196	149	.VS.....	.VS.....
	197	150	.V.....A...	.V.....A...
	198	151	..S....K....	..S....K....
	199	152	..S.....	..S.....
	200	153	..S....K....	..S....K....
	201	154	..S.....A...	..S.....A...
	202	155	..S.....T....	..S.....T....
	203	156	..S.....D....	..S.....D....
	204	157K..A...K..A...
	205	158A...A...
	206	159KA...KA...
	207	160AT...AT...
	208	161A..DA..D
	209	162	LVS...KLKAT.D	LVS...KLKAT.D
rabbit β -TM*	210	163	Q.A.....	Q.A.....
	217	164	.QA.....	.QA.....
	218	165	QQA...KLKGT.D	QQA...KLKGT.D

Table 3.1
Combinatorial Substitutions
Region 1 (continued)

	Spot	peptide	#12	#4
salmonTM1*	219	166	.I.....	.I.....
	229	167
	230	168	...M.....	...M.....
	231	169	LI.....	LI.....
	232	170	L.Q.....
	233	171	L..M.....	L..M.....
	234	172	.IQ.....	.IQ.....
	235	173	.I..M.....	.I..M.....
	236	174	.I...K.....	.I...K.....
	237	175	.I...L.....	.I...L.....
	238	176	.I...K.....	.I...K.....
	239	177	.I...G.....	.I...G.....
	240	178	.I...T.....	.I...T.....
	241	179	.I.....D	.I.....D
	242	180	..QM.....	..QM.....
	243	181	..Q...K.....	..Q...K.....
	244	182
	245	183	..Q...K.....	..Q...K.....
	246	184	..Q...G.....	..Q...G.....
	247	185	..Q...T.....	..Q...T.....
	248	186D.....D.....
	249	187	...M..K.....	...M..K.....
	250	188	...M..L.....	...M..L.....
	251	189	...M...K.....	...M...K.....
	252	190	...M...G.....	...M...G.....
	253	191	...M...T.....	...M...T.....
	254	192	...M.....D	...M.....D
	255	193	LIQM...KLKGT.D	LIQM...KLKGT.D
salmonTM2*	256	194	.L.....	.L.....
	265	195N.....N.....
	266	196	LL.....	LL.....
	267	197	L...N.....	L...N.....
	268	198	.LS.....	.LS.....
	269	199	.L...N.....	.L...N.....
	270	200	.L...L.....	.L...L.....
	271	201	.L...K.....	.L...K.....
	272	202	.L...G.....	.L...G.....
	273	203	.L...T.....	.L...T.....
	274	204	.L.....D	.L.....D
	275	205	..S...N.....	..S...N.....
	276	206NL.....NL.....
	277	207N.K.....N.K.....
	278	208N..G.....N..G.....
	279	209N...T.....N...T.....
	280	210N.....DN.....D
	281	211	LLS...NLKGT.D	LLS...NLKGT.D

*: mutated peptides resulting from sequence differences of Pen a 1 with this tropomysin.

Table 3.2
Combinatorial substitutions
region 2

	Spot	peptide	#6	#10	#18 (not done)	Spot	peptide	#12
Ser = 10	1	1	ALNRRIQLLEEDLER	ALNRRIQLLEEDLER	ALNRRIQLLEEDLER	1	1	ALNRRIQLLEEDLER
	2	2I.....I.....I.....	2	2I.....
S116QTM*	13	3	ALNRRIQLLEEDLER	ALNRRIQLLEEDLER	ALNRRIQLLEEDLER	3	13	ALNRRIQLLEEDLER
	14	4	S.....	S.....			
	15	5V.....V.....V.....	4	14V.....
	16	6E.....E.....E.....	5	15E.....
	17	7D.....D.....D.....	6	16D.....
	18	8	S.....V.....	S.....V.....	S.....V.....			
	19	9	S.....Z.....	S.....Z.....	S.....Z.....			
	20	10	S.....D.....	S.....D.....	S.....D.....			
	21	11V..Z..V..Z..V..Z..	7	17V..Z..
	22	12V...D..V...D..V...D..	8	18V...D..
	23	13E..D..E..D..E..D..	9	19E..D..
	24	14	S.....V..Z..	S.....V..Z..	S.....V..Z..			
	25	15	S.....V...D..	S.....V...D..	S.....V...D..			
	26	16	S.....E..D..	S.....E..D..	S.....E..D..			
	27	17V..Z.D.V..Z.D.V..Z.D.	10	20V..Z.D.
	28	18	S.....V..Z.D.	S.....V..Z.D.	S.....V..Z.D.			
S116QTM*	29	19F.....F.....F.....	11	21F.....
	30	20	S.....F.....	S.....F.....	S.....F.....			
	31	21F..E...F..E...F..E...	12	22F..E...
	32	22F...D..F...D..F...D..	13	23F...D..
	33	23	S.....F..E...	S.....F..E...	S.....F..E...			
	34	24	S.....F...D..	S.....F...D..	S.....F...D..			
	35	25F..E.D.F..E.D.F..E.D.	14	24F..E.D.
	36	26	S.....F..E.D.	S.....F..E.D.	S.....F..E.D.			
S116QTM*	37	27	G.....	G.....			
	38	28	G.....V.....V.....	G.....V.....			
	39	29	G.....Z.....Z.....	G.....Z.....			
	40	30	G.....D.....	G.....D.....	G.....D.....			
	41	31	G.....V..Z..	G.....V..Z..	G.....V..Z..			
	42	32	G.....V...D..	G.....V...D..	G.....V...D..			
	43	33	G.....E..D..	G.....E..D..	G.....E..D..			
	44	34	G.....V..Z.D.	G.....V..Z.D.	G.....V..Z.D.			

*: mutated peptides resulting from sequence differences of Pen a 1 with this tropomyosin.

Table 3.3
Combinatorial Substitutions
region 3 (epitope 3a)

Table 3.3 Combinatorial Substitutions region 3 (epitope 3a)																	
	#12				#1				#5				#18		#10		
	spot	pepti de			spot	pepti de			spot	pepti de			spot	pepti de			
Per a 7	1	1	RSLSDEPRM	1	1	1	LSDEPRM	1	1	1	1	SDPRM					
	2	2														
	3	3														
	4	4														
	5	5	KG.....		2	2	.A.....	2	2	2	2	A.....					
	6	6														
	7	7														
	8	8	KG.A.....														
Der p10	13	9	RSLSDEPRM	13	3	3	LSDEPRM	13	3	3	3	SDPRM	1	1	1		
	14	10		4	4	I.....										
	15	11		5	5										
				6	6										
				7	7										
				8	8	IT.....										
				9	9	I.....										
				10	10	I.....										
				11	11	T.....										
				12	12	T.....										
				13	13	T.....										
				14	14	IT.....										
			15	15	IT.....											
			16	16	I.....											
			17	17	T.....											
			18	18	IT.....											
			19	19											
			20	20	IT.....											
			21	21											
			22	22											
			23	23											
			24	24	IT.....											
			25	25	IT.....											
			26	26	I.....											
			27	27	T.....											
			28	28	IT.....											
			29	29											
			30	30	IT.....											
			31	31											
			32	32	IT.....											
			33	33											
			34	34	IT.....											
			35	35											
			36	36	IT.....											
			37	37											
			38	38	IT.....											
			39	39											
			40	40	IT.....											
			41	41											
			42	42	IT.....											
			43	43											
			44	44	IT.....											
			45	45											
			46	46	IT.....											
			47	47											
			48	48	IT.....											
			49	49											
			50	50	IT.....											
			51	51											
			52	52	IT.....											
			53	53											
			54	54	IT.....											
			55	55											
			56	56	IT.....											
			57	57											
			58	58	IT.....											
			59	59											
			60	60	IT.....											
			61	61											
			62	62	IT.....											
			63	63											
			64	64	IT.....											
			65	65											
			66	66	IT.....											
			67	67											
			68	68	IT.....											
			69	69											
			70	70	IT.....											
			71	71											
			72	72	IT.....											
			73	73											
			74	74	IT.....											
			75	75											
			76	76	IT.....											
			77	77											
			78	78	IT.....											
			79	79											
			80	80	IT.....											
			81	81											
			82	82	IT.....											
			83	83											
			84	84	IT.....											
			85	85											
			86	86	IT.....											
			87	87											
			88	88	IT.....											
			89	89											
			90	90	IT.....											
			91	91											
			92	92	IT.....											
			93	93											
			94	94	IT.....											
			95	95											
			96	96	IT.....											
			97	97											
			98	98	IT.....											
			99	99											
			100	100	IT.....											
			101	101											
			102	102	IT.....											
			103	103											
			104	104	IT.....											
			105	105											
			106	106	IT.....											
			107	107											
			108	108	IT.....											
			109	109											
			110	110	IT.....											
			111	111											
			112	112	IT.....											
			113	113											
			114	114	IT.....											
			115	115											
			116	116	IT.....											
			117	117											
			118	118	IT.....											
			119	119											
			120	120	IT.....											
			121	121											
			122	122	IT.....											
			123	123											
			124	124	IT.....											
			125	125											
			126	126	IT.....											
			127	127											
			128	128	IT.....											
			129	129											
			130	130	IT.....											
			131	131											
			132	132	IT.....											
			133	133											
			134	134	IT.....											
			135	135											
			136	136	IT.....											
			137	137											
			138	138	IT.....											
			139	139											
			140	140	IT.....											
			141	141											
			142	142	IT.....											
			143	143											
			144	144	IT.....											
			145	145											
			146	146	IT.....											
			147	147											
			148	148	IT.....											
			149	149											
			150	150	IT.....											
			151	151											
			152	152	IT.....											
			153	153											
			154	154	IT.....											
			155	155											
																

Table 3.3
Combinatorial Substitutions
region 3 (epitope 3a (continued))

#12				#1				#5				#18				#10
spot	pepti de			spot	pepti de			spot	pepti de			spot	pepti de			
25	13	..K..	..K..	37	19	..K..	..K..	25	11	..K..	..K..	13	5	..K..	..K..	
26	14	..K..	..K..	38	20	..K..	..K..	26	12	..K..	..K..	14	6	..K..	..K..	
27	15	..K..	..K..	39	21	..K..	..K..	27	13	..K..	..K..	15	7	..K..	..K..	
28	16	..K..	..K..	40	22	..K..	..K..	28	14	..K..	..K..	16	8	..K..	..K..	
29	17	..K..	..K..	41	23	..K..	..K..	29	15	..K..	..K..	17	9	..K..	..K..	
30	18	..K..	..K..	42	24	..K..	..K..									
31	19	..K..	..K..	43	25	..K..	..K..									
32	20	..K..	..K..	44	26	..K..	..K..									
33	21	..K..	..K..													
34	22	..K..	..K..													
35	23	..K..	..K..													
36	24	..K..	..K..													
37	25	..K..	..K..													
38	26	..K..	..K..													
39	27	..K..	..K..													
40	28	..K..	..K..													
41	29	..K..	..K..													
42	30	..K..	..K..													
43	31	..K..	..K..													
44	32	..K..	..K..													
45	33	..K..	..K..													
46	34	..K..	..K..													
47	35	..K..	..K..													
48	36	..K..	..K..													
49	37	..K..	..K..													
50	38	..K..	..K..													
51	39	..K..	..K..													
52	40	..K..	..K..													
53	41	..K..	..K..													
54	42	..K..	..K..													
55	43	..K..	..K..													
56	44	..K..	..K..													
57	45	..K..	..K..													
58	46	..K..	..K..													
59	47	..K..	..K..													
60	48	..K..	..K..													
61	49	..K..	..K..													
62	50	..K..	..K..													
63	51	..K..	..K..													
64	52	..K..	..K..													
65	53	..K..	..K..													
66	54	..K..	..K..													
67	55	..K..	..K..													
68	56	..K..	..K..													
69	57	..K..	..K..													
70	58	..K..	..K..													
71	59	..K..	..K..													
72	60	..K..	..K..													
73	61	..K..	..K..													
74	62	..K..	..K..													
75	63	..K..	..K..													
76	64	..K..	..K..													
77	65	..K..	..K..													
78	66	..K..	..K..													
79	67	..K..	..K..													
80	68	..K..	..K..													
81	69	..K..	..K..													
82	70	..K..	..K..													
83	71	..K..	..K..													
84	72	..K..	..K..													
85	73	..K..	..K..													
86	74	..K..	..K..													
87	75	..K..	..K..													
88	76	..K..	..K..													
89	77	..K..	..K..													
90	78	..K..	..K..													
91	79	..K..	..K..													
92	80	..K..	..K..													
93	81	..K..	..K..													
94	82	..K..	..K..													
95	83	..K..	..K..													
96	84	..K..	..K..													
97	85	..K..	..K..													
98	86	..K..	..K..													
99	87	..K..	..K..													
100	88	..K..	..K..													
101	89	..K..	..K..													
102	90	..K..	..K..													
103	91	..K..	..K..													
104	92	..K..	..K..													
105	93	..K..	..K..													
106	94	..K..	..K..													
107	95	..K..	..K..													
108	96	..K..	..K..													
109	97	..K..	..K..													
110	98	..K..	..K..													
111	99	..K..	..K..													
112	100	..K..	..K..													

GalpαTM

Table 3.3
Combinatorial Substitutions
region 3 (epitope 3a) (continued)

	#12			#1			#5			#18			#10
	spot	pepti da		spot	pepti da		spot	pepti da		spot	pepti da		
Gal β TM ^a	49	29	..M.....	73	49	M.....	49	27L	37	21L..	
	50	30	..AM.....	74	50							
	51	31	..MK.....	75	51							
	52	32	..M....K.	76	52	MK.....							
				77	53	M....K.....							
				78	54	M....E.....							
				79	55							
				80	56	M.....Q..							
				81	57							
				82	58	..K....L...		28	K....L				
				83	59	..K....M		29K..L	38	22K..L..	...K..L..
				84	60							
				85	61K....M		30EL	39	23EL..	...EL..
				86	62EL...							
				87	63E...M							
				88	64LQ...				40	24LQ.	...LQ.
				89	65							
				90	66Q..M							
	53	33	..AMK.....				53	31	K....K..L	41	25K..EL..	...K..EL..
	54	34	..AM....K.				54	32	K....EL	42	26K..LQ.	...K..LQ.
	55	35	..MK....K.				55	33K..EL	43	27ELQ.	...ELQ.
	56	36	..AMK....K.				56	34	K....K..EL	44	28K..ELQ.	...K..ELQ.

Table 3.3 Combinatorial Substitutions Region 3 (epitope 3a) (continued)											
#12				#1		#5		#18		#10	
spot	pepti de			spot	pepti de	spot	pepti de	spot	pepti de	spot	pepti de
61	37	..S.....		97	68	98	69	49	29		
62	38	.AS.....									
63	39	..SK.....		99	70	100	71				
64	40	..S....K.		101	72	102	73				
				103	74	103	75				
				105	76	106	77				
				107	78	108	79	50	30		
				109	80	110	81	51	31		
				111		112		52	32		
								53	34		

Table 3.4 Combinatorial Substitutions region 3 (epitope 3b)						
#4				#6		
	spots	peptide		spots	peptide	
Der p 10*	1	1				
	2	2				
	3	3G.....			
	4	4G.....M			
	5	5EG.....			
	6	6E.....M			
	7	7G.....M			
	8	8EG.....M			
Gal α TM*	13	9		1	1	LENQLKEA
	14	10				
	15	11				
	16	12				
	17	13Q.....	2	2
	18	14I.....	3	3
	19	15K.....			
	20	16H.....			
	21	17				
	22	18				
	23	19	...K...Q.....			
	24	20				
	25	21				
	26	22	...K.....H			
	27	23				
	28	24	...E...Q.....			
	29	25	...E...I.....			
	30	26				
	31	27	...E.....H			
	32	28IQ.....			
	33	29I.....			
	34	30				
	35	31I.....H	4	4	Q.I.....
	36	32Q.I.....			
	37	33Q.....K.			
	38	34Q.....H			
	39	35I.....K.			
	40	36I.....H			
	41	37KH.....			
	42	38	...K.EIQ.I.....KH			
Gal β TM*	49	39				
	50	40M.....	13	5	...M.....
	51	41				
	52	42				
	53	43				
	54	44	...E...M.....			
	55	45				
	56	46L..M.....			
	57	47				
	58	48L.....H			
	59	49Q.M.....	14	5	Q.M.....
	60	50M.....K.			
	61	51M.....H			
	62	52	...K.ELQ.M.....KH			
SaltTM1*	73	53D.....	25	7	.D.....
	74	54	...K...D.....	26	8	QD.....
	75	55E..D.....	27	9	.DI.....
	76	56L.D.....	28	10	QDI.....
	77	57L..I.....			
	78	58QD.....			
	79	59DI.....			
	80	60D.....K.			
	81	61D.....H			
	82	62	...K.ELQDI.....KH			

*: mutated peptides resulting from sequence differences of Pen a 1 with this tropomyosin

Table 3.5 (continued)
Combinatorial Substitution Analysis
Epitope 5a

	spot	peptide	#2	spot	peptide	#4	spot	peptide	#10	spot	peptide	#18
Catalytic	37	17	...T...	37	9	...T...	49	33	...T...			
	38	18	...T...			...T...	50	34	...T...			
	39	19	...T...			...T...	51	35	...T...			
	40	20	...T...			...T...	52	36	...T...			
	41	21	...T...	38	10	...T...	53	37	...T...			
	42	22	...T...	39	11	...T...	54	38	...T...			
	43	23	...T...			...T...	55	39	...T...			
	44	24	...T...			...T...	56	40	...T...			
	45	25	...T...			...T...	57	41	...T...			
			...T...			...T...	58	42	...T...			
	46	26	...T...			...T...	59	43	...T...			
	47	27	...T...			...T...	60	44	...T...			
	48	28	...T...			...T...	61	45	...T...			
	49	29	...T...			...T...	62	46	...T...			
	50	30	...T...			...T...	63	47	...T...			
			...T...			...T...	64	48	...T...			
	51	31	...T...	40	12	...T...	65	49	...T...			
	52	32	...T...			...T...	66	50	...T...			
	53	33	...T...			...T...	67	51	...T...			
	54	34	...T...			...T...	68	52	...T...			
			...T...			...T...	69	53	...T...			
	55	35	...T...			...T...	70	54	...T...			
	56	36	...T...			...T...	71	55	...T...			
	57	37	...T...			...T...	72	56	...T...			
	58	38	...T...			...T...	73	57	...T...			
	59	39	...T...			...T...	74	58	...T...			
			...T...			...T...	75	59	...T...			
	60	40	...T...			...T...	76	60	...T...			
			...T...			...T...	77	61	...T...			
			...T...			...T...	78	62	...T...			
			...T...			...T...	79	63	...T...			
			...T...			...T...	80	64	...T...			
			...T...			...T...	81	65	...T...			
			...T...			...T...	82	66	...T...			
			...T...			...T...	83	67	...T...			
			...T...			...T...	84	68	...T...			
			...T...			...T...	85	69	...T...			
			...T...			...T...	86	70	...T...			
			...T...			...T...	87	71	...T...			
			...T...			...T...	88	72	...T...			
			...T...			...T...	89	73	...T...			
			...T...			...T...	90	74	...T...			
			...T...			...T...	91	75	...T...			
			...T...			...T...	92	76	...T...			
			...T...			...T...	93	77	...T...			
			...T...			...T...	94	78	...T...			
			...T...			...T...	95	79	...T...			
			...T...			...T...	96	80	...T...			

Table 3.5 (continued)
torial Substitution Analysis
Epitope 5b

	spot	peptide	#2	spot	peptide	#4	spot	peptide	#10	spot	peptide	#18
Der 37°	1	41	...	1	13	...	1	81	...	1	1	...
	2	42	...	2	14	...	2	82	...	2	2	...
	3	43	...	3	15	...	3	83	...	3	3	...
	4	44	...	4	16	...	4	84	...	4	4	...
	5	45	...	5	17	...	5	85	...	5	5	...
	6	46	...	6	18	...	6	86	...	6	6	...
	7	47	...	7	19	...	7	87	...	7	7	...
	8	48	...	8	20	...	8	88	...	8	8	...
Der p 10°	11	49	...	11	21	...	11	89	...	11	9	...
	12	50	...	12	22	...	12	90	...	12	10	...
	61	51	...	49	23	...	97	91	...	13	11	...
	62	52	...	50	24	...	98	92	...	14	12	...
Galg α'1M°	63	53	...	51	25	...	99	93	...	15	13	...
	64	54	...	52	26	...	100	94	...	16	14	...
	65	55	...	53	27	...	101	95	...	17	15	...
	66	56	...	54	28	...	102	96	...	18	16	...
	67	57	...	55	29	...	103	97	...	19	17	...
	68	58	...	56	30	...	104	98	...	20	18	...
		
		

Table 3.5 (continued) Combinatorial Substitution Analysis Epitope 5c									
	spot	peptide	#1	spot	peptide	#2	spot	peptide	#18
Pen a 7*	13	31	...	14	32	...	15	33	...
	16	34	...	17	35	...	18	36	...
	19	37	...	20	38	...	21	39	...
	22	40	...	23	41	...	24	42	...
	25	43	...	26	44	...	27	45	...
	28	46	...	29	47	...	30	48	...
	31	49	...	32	50	...	33	51	...
	34	52	...	35	53	...	36	54	...
	37	55	...	38	56	...	39	57	...
	40	58	...	41	59	...	42	60	...
Herp 10*	43	61	...	44	62	...	45	63	...
	46	64	...	47	65	...	48	66	...
	49	67	...	50	68	...	51	69	...
	52	70	...	53	71	...	54	72	...
	55	73	...	56	74	...	57	75	...
	58	76	...	59	77	...	60	78	...
	61	79	...	62	80	...	63	81	...
	64	82	...	65	83	...	66	84	...
	67	85	...	68	86	...	69	87	...
	70	88	...	71	89	...	72	90	...
Galg α1N1*	73	91	...	74	92	...	75	93	...
	76	94	...	77	95	...	78	96	...
	79	97	...	80	98	...	81	99	...
	82	100	...	83	101	...	84	102	...
	85	103	...	86	104	...	87	105	...
	88	106	...	89	107	...	90	108	...
	91	109	...	92	110	...	93	111	...
	94	112	...	95	113	...	96	114	...
	97	115	...	98	116	...	99	117	...
	100	118	...	101	119	...	102	120	...
SubTMD*	103	121	...	104	122	...	105	123	...
	106	124	...	107	125	...	108	126	...
	109	127	...	110	128	...	111	129	...
	112	130	...	113	131	...	114	132	...
	115	133	...	116	134	...	117	135	...
	118	136	...	119	137	...	120	138	...
	121	139	...	122	140	...	123	141	...
	124	142	...	125	143	...	126	144	...
	127	145	...	128	146	...	129	147	...
	130	148	...	131	149	...	132	150	...

*: mutated peptides resulting from sequence differences of Pen a 1 with this tropomyosin

	Pen a 1		Region 1		Region 2		Region 3		Region 4		Region 5	
tropomyosin	Ide	Sim	Ide	Sim	Ide	Sim	Ide	Sim	Ide	Sim	Ide	Sim
Hom a TMf	98	98	100	100	100	100	100	100	100	100	100	100
Hom a Tms	93	96	46	80	100	100	100	100	100	100	100	100
Pan s 1	98	98	100	100	100	100	100	100	95	95	100	100
Per a 7	82	90	60	93	100	100	80	87	100	100	81	92
Der f.10	81	89	60	86	95	100	80	100	100	100	89	94
Der p 10	81	89	60	86	95	100	73	90	100	100	89	94
Dro m TM	70	87	46	93	100	100	87	87	95	100	68	81
Myt e TM	57	75	53	73	52	80	40	53	57	80	76	86
Onc v TM	70	83	53	80	80	90	53	73	90	95	81	94
Sch m TM	60	74	26	60	71	76	53	67	90	90	65	86
Gal g TM	58	72	33	53	71	85	47	67	76	85	44	63
Ory c TM	56	72	33	60	71	85	47	67	71	80	44	63

⁴
Table 4: Sequence identities (Ide) and similarities (Sim) among Pen a 1, Pen a 1 epitopes and other allergenic and non-allergenic tropomyosins (TM)

Table 4

Table S (a)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 1			
	mutated amino acid positions that never show any IgE reactivity	mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence
Pen a 1 sequence	Pen a 1 43-55	VHNLQKRMQQLLEN	VHNLQKRMQQLLEN
mutated sequences containing 1 substitution	Pen a 1 44 ¹	.I.....	I.....
	Pen a 1 46 ¹	...M.....	I.....
	Pen a 1 46 ²	...T.....	.K.....
	Pen a 1 47 ¹H.....	.Q.....
	Pen a 1 53 ¹V..	..S.....
	Pen a 1 43 ¹	A.....	..I.....
	Pen a 1 44 ¹	.V.....	..G.....
	Pen a 1 51 ¹K....K.....
	Pen a 1 53 ²T..G....
	Pen a 1 44 ²	.R.....A....
	Pen a 1 49 ¹K.....I..
	Pen a 1 44 ²	.L.....D
	Pen a 1 49 ²N.....	
	Pen a 1 43 ²	Q.....	
	Pen a 1 43 ¹ 44 ¹	IR.....	Q.A.....
	Pen a 1 43 ¹ 46 ¹	I..T.....	Q.....I.....
mutated sequences containing 2 substitutions	Pen a 1 43 ¹ 47 ¹	I...H.....	L.S.....
	Pen a 1 43 ¹ 53 ¹	I.....V..	L.Q.....
	Pen a 1 44 ¹ 45 ¹	.RI.....	L.A.....
	Pen a 1 44 ¹ 46 ¹	.R.T.....	L.....L.....
	Pen a 1 44 ¹ 47 ¹	.R..H.....	L.....A....
	Pen a 1 44 ¹ 49 ¹	.R....K.....	L.....D
	Pen a 1 44 ¹ 53 ¹	.R.....V..	I.I.....
	Pen a 1 45 ¹ 46 ¹	..IT.....	I.....K.....
	Pen a 1 45 ¹ 47 ¹	..I.H.....	A.....I.....
	Pen a 1 45 ¹ 53 ¹	..I.....V..	.V.....I.....
	Pen a 1 46 ¹ 47 ¹	...TH.....	.Q.....L.....
	Pen a 1 46 ¹ 49 ¹	...T..K.....	.L.....H.....

Table 5(a)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 1 (continued)				
Pen a 1 sequence	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
mutated sequences containing 2 substitutions	Pen a 1 43-55	VIRLQKRMQQLN	Pen a 1 43-55	VIRLQKRMQQLN
	Pen a 1 46 ⁵ 53 ⁵	...T.....V..	Pen a 1 44 ⁵ 50 ⁵	..L.....L.....
	Pen a 1 47 ⁵ 49 ⁵H.K.....	Pen a 1 45 ⁵ 49 ⁵	..S...K.....
	Pen a 1 47 ⁵ 53 ⁵H.....V..	Pen a 1 45 ⁵ 52 ⁵	..S.....G...
	Pen a 1 49 ⁵ 53 ⁵K...V..	Pen a 1 45 ⁵ 52 ⁵	..S.....A...
	Pen a 1 43 ⁵ 44 ⁵	AR.....	Pen a 1 45 ⁵ 53 ⁵	..S.....I...
	Pen a 1 43 ⁵ 45 ⁵	A.B.....	Pen a 1 45 ⁵ 49 ⁵	..O...K.....
	Pen a 1 43 ⁵ 49 ⁵	A.....K.....	Pen a 1 45 ⁵ 51 ⁵	..O.....K....
	Pen a 1 43 ⁵ 53 ⁵	A.....I..	Pen a 1 45 ⁵ 52 ⁵	..Q.....G...
	Pen a 1 44 ⁵ 45 ⁵	.RS.....	Pen a 1 45 ⁵ 49 ⁵	..I...K.....
	Pen a 1 44 ⁵ 49 ⁵	.R....K.....	Pen a 1 45 ⁵ 49 ⁵	..G...K.....
	Pen a 1 44 ⁵ 50 ⁵	.R.....I.....	Pen a 1 45 ⁵ 49 ⁵	..A...K.....
	Pen a 1 44 ⁵ 53 ⁵	.R.....T..	Pen a 1 45 ⁵ 51 ⁵	..A.....K....
	Pen a 1 49 ⁵ 53 ⁵K...I..	Pen a 1 45 ⁵ 52 ⁵	..A.....G...
	Pen a 1 44 ⁵ 45 ⁵	.RA.....	Pen a 1 45 ⁵ 53 ⁵	..A.....I...
	Pen a 1 44 ⁵ 49 ⁵	.R....K.....	Pen a 1 46 ⁵ 53 ⁵	..M.....T...
	Pen a 1 44 ⁵ 50 ⁵	.R.....I.....	Pen a 1 49 ⁵ 50 ⁵NL.....
	Pen a 1 44 ⁵ 53 ⁵	.R.....I..	Pen a 1 49 ⁵ 50 ⁵KL.....
	Pen a 1 49 ⁵ 53 ⁵K...I..	Pen a 1 49 ⁵ 50 ⁵KI.....
	Pen a 1 43 ⁵ 44 ⁵	LV.....	Pen a 1 49 ⁵ 52 ⁵K...A...
	Pen a 1 43 ⁵ 49 ⁵	L.....K.....	Pen a 1 49 ⁵ 55 ⁵K.....D
	Pen a 1 43 ⁵ 51 ⁵	L.....K.....	Pen a 1 50 ⁵ 51 ⁵LK.....
	Pen a 1 43 ⁵ 52 ⁵	L.....G...	Pen a 1 50 ⁵ 52 ⁵L.G...
	Pen a 1 43 ⁵ 53 ⁵	L.....T...	Pen a 1 50 ⁵ 53 ⁵I...I...
	Pen a 1 44 ⁵ 45 ⁵	.VA.....	Pen a 1 51 ⁵ 55 ⁵K...D
	Pen a 1 44 ⁵ 49 ⁵	.V.....K.....	Pen a 1 52 ⁵ 55 ⁵G...D
	Pen a 1 44 ⁵ 51 ⁵	.V.....K.....	Pen a 1 52 ⁵ 55 ⁵A...D
	Pen a 1 44 ⁵ 52 ⁵	.V.....G...		
	Pen a 1 44 ⁵ 53 ⁵	.V.....T...		

Table 5(a)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 1 (continued)			
	mutated amino acid positions that never show any IgE reactivity	sequence	mutated amino acid positions that show reduced and/or abolished IgE reactivity
Pen a 1 sequence	sequence name	sequence	sequence
mutated sequences containing 2 substitutions	Pen a 1 43-55	VHNLQKRRQOLEN	Pen a 1 43-55
	Pen a 1 44 ^a 55 ^a	.V.....D	VHNLQKRRQOLEN
	Pen a 1 45 ^a 53 ^r	..A.....T..	
	Pen a 1 49 ^a 51 ^aK.K....	
	Pen a 1 49 ^a 52 ^aK..G...	
	Pen a 1 49 ^a 53 ^rK...T..	
	Pen a 1 50 ^a 53 ^rL..T..	
	Pen a 1 51 ^a 52 ^aKG...	
	Pen a 1 51 ^a 53 ^rK.T..	
	Pen a 1 52 ^a 53 ^rGT..	
	Pen a 1 53 ^r 55 ^aT.D	
	Pen a 1 43 ^a 44 ^a	QQ.....	
	Pen a 1 43 ^a 45 ^a	Q.G.....	
	Pen a 1 43 ^a 49 ^a	Q.....K.....	
	Pen a 1 43 ^a 51 ^a	Q.....K....	
	Pen a 1 43 ^a 52 ^a	Q.....G...	
	Pen a 1 43 ^a 53 ^r	Q.....T..	
	Pen a 1 43 ^a 55 ^a	Q.....D	
	Pen a 1 44 ^a 45 ^a	.QG.....	
	Pen a 1 44 ^a 49 ^a	.Q.....K.....	
	Pen a 1 44 ^a 51 ^a	.Q.....K....	
	Pen a 1 44 ^a 52 ^a	.Q.....G...	
	Pen a 1 44 ^a 53 ^r	.Q.....T..	
	Pen a 1 44 ^a 55 ^a	.Q.....D	
	Pen a 1 45 ^a 51 ^a	..G.....K....	
	Pen a 1 45 ^a 52 ^a	..G.....G....	
	Pen a 1 45 ^a 53 ^r	..G.....T..	
	Pen a 1 44 ^a 45 ^a	.VS.....	
	Pen a 1 44 ^a 52 ^a	.V.....A...	

Table 5 (a)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 1 (continued)			
	mutated amino acid positions that never show any IgE reactivity	sequence name	sequence
Pen a 1 sequence	Pen a 1 43-55	VHNLQKRNQOLEN	VHNLQKRNQOLEN
	Pen a 1 45 ^a 49 ^a	..S...K.....	
mutated sequences containing 2 substitutions	Pen a 1 45 ^a 51 ^a	..S...K.....	
	Pen a 1 45 ^a 53 ^a	..S...K.....	
	Pen a 1 51 ^a 52 ^a	..S...K.....	
	Pen a 1 52 ^a 53 ^a	..S...K.....	
	Pen a 1 44 ^a 45 ^a	..S...K.....	
	Pen a 1 43 ^a 44 ^a	..S...K.....	
	Pen a 1 43 ^a 46 ^a	..S...K.....	
	Pen a 1 44 ^a 45 ^a	..S...K.....	
	Pen a 1 44 ^a 46 ^a	..S...K.....	
	Pen a 1 44 ^a 49 ^a	..S...K.....	
	Pen a 1 44 ^a 51 ^a	..S...K.....	
	Pen a 1 44 ^a 52 ^a	..S...K.....	
	Pen a 1 44 ^a 53 ^a	..S...K.....	
	Pen a 1 44 ^a 55 ^a	..S...K.....	
	Pen a 1 45 ^a 46 ^a	..S...K.....	
	Pen a 1 45 ^a 53 ^a	..S...K.....	
	Pen a 1 46 ^a 49 ^a	..S...K.....	
	Pen a 1 46 ^a 50 ^a	..S...K.....	
	Pen a 1 46 ^a 51 ^a	..S...K.....	
	Pen a 1 46 ^a 52 ^a	..S...K.....	
	Pen a 1 46 ^a 55 ^a	..S...K.....	
	Pen a 1 43 ^a 44 ^a	..S...K.....	
	Pen a 1 43 ^a 49 ^a	..S...K.....	
	Pen a 1 44 ^a 45 ^a	..S...K.....	
	Pen a 1 44 ^a 49 ^a	..S...K.....	
	Pen a 1 44 ^a 51 ^a	..S...K.....	
	Pen a 1 44 ^a 52 ^a	..S...K.....	

Table 5(a)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope I (continued)				
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence mutated sequences containing 2 substitutions	Pen a 1 43-55	VIIII-QRRMQQLEN	Pen a 1 43-55	VIIII-QRRMQQLEN
	Pen a 1 44 ^a 53 ^r	.L.....T..		
	Pen a 1 44 ^a 55 ^p	.L.....D		
	Pen a 1 45 ^a 49 ^m	..S...N.....		
	Pen a 1 49 ^m 51 ^aN.K....		
	Pen a 1 49 ^m 52 ^mN..G...		
	Pen a 1 49 ^m 53 ^rN...T..		
	Pen a 1 49 ^m 55 ^pN.....U		

Table 5 (b)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 2			
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity
	sequence name	sequence	sequence
unmodified Pen a 1 sequence	Pen a 1 87-101	ALNRRQLLEEDLER	ALNRRQLLEEDLER
mutated sequences containing 1 substitution	Pen a 1 95 ^vV.....	G.....
		I.....
		F.....
		E.....
		D.....
		S.....
		S.....
mutated sequences containing 2 substitutions	Pen a 1 87 ^s 95 ^v	S.....V.....F.....D.....
	Pen a 1 95 ^v 98 ^tV..E...S.....E....
	Pen a 1 95 ^v 100 ^bV....D.S.....D....
	Pen a 1 98 ^s 100 ^bE.D.F.....D....
	Pen a 1 87 ^s 95 ^r	S.....F.....	
	Pen a 1 95 ^r 98 ^tF..E...	
	Pen a 1 87 ^s 95 ^v	G.....V.....	
	Pen a 1 87 ^s 98 ^t	G.....E...	
	Pen a 1 87 ^s 100 ^b	G.....D.	
	Pen a 1 87 ^s 95 ^v 98 ^t	G.....V..E...	
	Pen a 1 87 ^s 95 ^v 100 ^b	G.....V....D.	
	Pen a 1 87 ^s 95 ^v 98 ^t	S.....V..E...	
	Pen a 1 87 ^s 98 ^t 100 ^b	S.....E.D.	
	Pen a 1 95 ^v 98 ^t 100 ^bV..E.D.	
	Pen a 1 87 ^s 95 ^r 100 ^b	S.....F..E...	
mutated sequences containing 3 substitutions	Pen a 1 87 ^s 95 ^r 98 ^t	S.....F....D.	
	Pen a 1 87 ^s 95 ^r 100 ^b	G.....V....D.	
	Pen a 1 95 ^r 98 ^t 100 ^b	G.....E.D.	
	Pen a 1 87 ^s 95 ^r 98 ^tV..E.D.	
	Pen a 1 87 ^s 95 ^r 100 ^b	S.....F..E...	
	Pen a 1 95 ^r 98 ^t 100 ^b	S.....F....D.	
	Pen a 1 87 ^s 95 ^r 98 ^t	G.....V..E...	
	Pen a 1 87 ^s 95 ^r 100 ^b	G.....E.D.	
	Pen a 1 95 ^r 98 ^t 100 ^b	G.....V....D.	
	Pen a 1 87 ^s 95 ^r 98 ^t 100 ^b	S.....F..E.D.	
mutated sequences containing 4 substitutions	Pen a 1 87 ^s 95 ^r 98 ^t 100 ^b	S.....V..E.D.	
	Pen a 1 87 ^s 95 ^r 98 ^t 100 ^b	S.....F..E.D.	

Table 5(c)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 3a			
mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence
mutated sequences containing 1 substitution	Pen a 1 133-147	RSLSDEERHDALEN	RSLSDEERHDALEN
			...T.....
			...K.....
			...A.....
		K.....
		E.....
		I...
		G...
		D.
		I
mutated sequences containing 2 substitutions	Pen a 1 133 ^h 134 ^h	KG.....	K...A.....
	Pen a 1 134 ^h 136 ^h	..G..A.....	..A.....K.....
	Pen a 1 134 ^h 135 ^h	..AS.....	..S.....L...
	Pen a 1 134 ^h 135 ^h	..AQ.....	..Q.....I...
	Pen a 1 134 ^h 135 ^h	..AM.....	..Q.....I
	Pen a 1 134 ^h 136 ^h	..A..K.....	..H.....K.....
	Pen a 1 135 ^h 136 ^h	..SK.....	..TP.....
	Pen a 1 135 ^h 140 ^h	..S....K.....	...K.....L...
	Pen a 1 135 ^h 142 ^h	..S.....E.....K..L...
	Pen a 1 135 ^h 144 ^h	..S.....Q...K..I...
	Pen a 1 135 ^h 145 ^h	..S.....D.K.....N
	Pen a 1 135 ^h 146 ^h	..S.....IK.....I
	Pen a 1 135 ^h 136 ^h	..QK.....E..D.
	Pen a 1 135 ^h 140 ^h	..Q....K.....L..U.
	Pen a 1 135 ^h 142 ^h	..Q.....E.....L..N
	Pen a 1 135 ^h 144 ^h	..Q.....Q...L..I
	Pen a 1 135 ^h 136 ^h	..MK.....I..I

Table 5(c)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 3a (continued)			
mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
sequence name	sequence	sequence name	sequence
Pen a 1 133-147	RSLSDEERHDALEH	Pen a 1 133-147	RSLSDEERHDALEH
Pen a 1 135 ^M 142 ^E	..M.....E....	Pen a 1 145 ^P 145 ^PQD.
Pen a 1 135 ^M 144 ^N	..M.....Q..	Pen a 1 145 ^P 146 ^IDI
Pen a 1 135 ^I 142 ^M	..I.....E....		
Pen a 1 135 ^I 143 ^E	..I.....G....		
Pen a 1 136 ^T 142 ^E	...T.....E....		
Pen a 1 136 ^T 143 ^N	...T.....G....		
Pen a 1 136 ^E 140 ^K	...K...K.....		
Pen a 1 136 ^K 142 ^E	...K.....E....		
Pen a 1 136 ^K 143 ^I	...K.....I....		
Pen a 1 136 ^E 144 ^N	...K.....Q..		
Pen a 1 136 ^K 145 ^N	...K.....D..		
Pen a 1 136 ^K 146 ^M	...K.....M		
Pen a 1 136 ^K 146 ^I	...K.....I		
Pen a 1 140 ^K 142 ^EK.E....		
Pen a 1 140 ^K 144 ^NK...Q..		
Pen a 1 140 ^K 145 ^NK....D.		
Pen a 1 142 ^N 143 ^IEL....		
Pen a 1 142 ^N 143 ^IEI....		
Pen a 1 142 ^N 143 ^NEG....		
Pen a 1 142 ^N 144 ^NE.Q..		
Pen a 1 142 ^N 146 ^ME...M		
Pen a 1 142 ^N 146 ^IE...I		
Pen a 1 143 ^I 144 ^NIQ..		
Pen a 1 143 ^I 144 ^NIQ..		
Pen a 1 144 ^N 146 ^MQ.M		
Pen a 1 144 ^N 146 ^IQ.I		

mutated sequences containing
2 substitutions

Table 5(c)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 3a (continued)			mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	Pen a 1 133-147	RSLSDERHDALEN	Pen a 1 133-147	RSLSDERHDALEN
	Pen a 1 133 ^S 134 ^S 136-A	KG...A.....		
	Pen a 1 134 ^A 135 ^S 136-K	.ASK.....		
	Pen a 1 134 ^A 135 ^S 140-K	.AS....K.....		
	Pen a 1 134 ^A 135 ^S 136-K 140-K*	.AQK....K.....		
	Pen a 1 134 ^A 135 ^S 136-K	.AQK.....		
	Pen a 1 134 ^A 135 ^S 140-K	.AQ....K.....		
	Pen a 1 134 ^A 135 ^M 136-K	.AMK.....		
	Pen a 1 134 ^A 135 ^M 140-K	.AM....K.....		
	Pen a 1 134 ^A 136 ^S 140-K	.A.K....K.....		
	Pen a 1 135 ^S 136 ^S 140-K	..SK....K.....		
	Pen a 1 135 ^S 136 ^S 140-K	..QK....K.....		
	Pen a 1 135 ^M 136 ^S 140-K	..MK....K.....		
	Pen a 1 135 ^S 136 ^S 142-E	..T.....E....		
	Pen a 1 135 ^S 136 ^S 143-G	..T.....G....		
	Pen a 1 135 ^S 142 ^S 143-Q	..I.....EG...		
mutated sequences containing 3 substitutions	Pen a 1 136 ^S 142 ^S 143-G	...T.....EG...		
	Pen a 1 136 ^S 140 ^S 142-U	...K....K.E....		
	Pen a 1 136 ^S 140 ^S 143-L	...K....K...L...		
	Pen a 1 136 ^S 140 ^S 143-I	...K....K...I...		
	Pen a 1 136 ^S 142 ^S 143-I	...K....K.EI...		
	Pen a 1 136 ^S 142 ^S 143-I	...K....K.EI...		
	Pen a 1 140 ^S 142 ^S 143-LK.EI...		
	Pen a 1 140 ^S 142 ^S 143-IK.EI...		
	Pen a 1 140 ^S 142 ^S 144-QK.E.Q...		
	Pen a 1 140 ^S 142 ^S 145-DK.E...D.		
	Pen a 1 140 ^S 143 ^S 144-QK...LQ...		

Table 5(c)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 3a (continued)			mutated amino acid positions that show reduced and/or abolished IgE reactivity		sequence
mutated amino acid positions that never show any IgE reactivity			sequence name	sequence	
Pen a 1 sequence	Pen a 1 133-147		RSLSDDEERDALEN	Pen a 1 133-147	RSLSDDEERDALEN
	Pen a 1 140 ⁵ 143 ¹ 145-D	K...L.D.		
	Pen a 1 140 ⁵ 143 ¹ 144-Q	K...IQ..		
mutated sequences containing 3 substitutions	Pen a 1 140 ⁵ 144 ⁹ 145-D	K...QD.		
	Pen a 1 142 ⁸ 143 ¹ 144-Q	ELQ..		
	Pen a 1 142 ⁸ 143 ¹ 145-D	EL.D.		
	Pen a 1 142 ⁸ 143 ¹ 144-Q	ELQ..		
	Pen a 1 142 ⁸ 144 ⁹ 145-D	E.QD.		
	Pen a 1 143 ¹ 144 ⁹ 145-D	LQD.		
	Pen a 1 134 ⁶ 135 ³ 136-K 140-K ²		.ASK....K.....		
mutated sequences containing 4 substitutions	Pen a 1 134 ⁶ 135 ³ 136-K 140-K ²		.AQK....K.....		
	Pen a 1 134 ⁶ 135 ³ 136-K 140-K ²		.AMK....K.....		
	Pen a 1 135 ³ 136 ¹ 142-E 143-Q ²		..IT.....EG...		
	Pen a 1 136 ⁵ 140 ⁵ 142-E 143-I ²		...K....K.EI...		
	Pen a 1 140 ⁵ 142 ⁸ 143-L 145-D ²	K.EL.D.		
	Pen a 1 140 ⁵ 142 ⁸ 143-L 144-Q ²	K.EIQ..		
	Pen a 1 140 ⁵ 142 ⁸ 144-Q 145-D ²	K.E.QD.		
	Pen a 1 140 ⁵ 143 ¹ 144-Q 145-D ²	K...LQD.		
	Pen a 1 142 ⁸ 143 ¹ 144-Q 145-D ²	ELQD.		

Table 5(d)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 3b				
mutated amino acid positions that never show any IgE reactivity			mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	peptide name	peptide sequence	peptide name	peptide sequence
unmodified Pen a 1 sequence	Pen a 1 139-153	ERMDALENQLKEARF	Pen a 1 139-153	ERMDALENQLKEARF
	Pen a 1 143 ^aG.....		
	Pen a 1 144 ^aQ.....		
mutated sequences containing 1 substitution	Pen a 1 145 ^aD.....		
	Pen a 1 153 ^aM		
	Pen a 1 153 ^aH		
mutated sequences containing 2 substitutions	Pen a 1 140 ^a 144 ^a	.K...Q.....	Pen a 1 140 ^a 153 ^a	.K.....H
	Pen a 1 140 ^a 145 ^a	.K....D.....	Pen a 1 142 ^a	...E.....
	Pen a 1 142 ^a 143 ^a	...EG.....	Pen a 1 142 ^a 144 ^a	...E.Q.....
	Pen a 1 142 ^a 145 ^a	...E..D.....	Pen a 1 142 ^a 153 ^a	...E.....H
	Pen a 1 142 ^a 146 ^a	...E...M.....	Pen a 1 143 ^a 144 ^aIQ.....
	Pen a 1 142 ^a 146 ^a	...E...I.....	Pen a 1 143 ^a 145 ^aL.D.....
	Pen a 1 142 ^a 153 ^a	...E.....M	Pen a 1 143 ^a 146 ^aL..I.....
	Pen a 1 143 ^a 146 ^aL..M.....	Pen a 1 143 ^a 153 ^aL.....H
	Pen a 1 143 ^a 146 ^aI..I.....	Pen a 1 143 ^a 144 ^aIO.....
	Pen a 1 143 ^a 153 ^aI.....H	Pen a 1 143 ^a 152 ^aI.....K.
	Pen a 1 143 ^a 153 ^aG.....M	Pen a 1 146 ^a 152 ^aM.....K.
	Pen a 1 144 ^a 145 ^aQD.....	Pen a 1 146 ^aM.....
	Pen a 1 144 ^a 146 ^aQ.M.....	Pen a 1 146 ^a 152 ^aI.....K.
	Pen a 1 144 ^a 146 ^aQ.I.....	Pen a 1 146 ^a 153 ^aI.....H
	Pen a 1 144 ^a 152 ^aQ.....K.		
	Pen a 1 144 ^a 153 ^aQ.....H		
	Pen a 1 145 ^a 146 ^aDI.....		
	Pen a 1 145 ^a 152 ^aD.....K.		
	Pen a 1 145 ^a 153 ^aD.....H		
	Pen a 1 146 ^a 153 ^aM.....H		
	Pen a 1 152 ^a 153 ^aKII		

Table 5(c)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5a				
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	penal 249-261	LQKEVDRIEDELV	penal 249-261	LQKEVDRIEDELV
	penal 255 ^aD.....	penal 250 ^a	.E.....
	penal 260 ^aV.....	penal 252 ^a	...T.....
	penal 261 ^aY.....	penal 252 ^a	...S.....
mutated sequences containing 1 substitution			penal 253 ^aI.....
	penal 250 ^a 252 ^a	.E.S.....	penal 250 ^a 252 ^a	.E.T.....
	penal 250 ^a 255 ^a	.E....D.....	penal 250 ^a 253 ^a	.E..I.....
	penal 250 ^a 260 ^a	.S.....V.....	penal 250 ^a 255 ^a	.E....D.....
	penal 252 ^a 255 ^a	...T..D.....	penal 252 ^a 253 ^a	...TI.....
	penal 252 ^a 260 ^a	...T.....V.....	penal 252 ^a 253 ^a	...SI.....
	penal 252 ^a 255 ^a	...S..D.....	penal 253 ^a 261 ^aI.....Y
	penal 252 ^a 261 ^a	...S.....Y		
	penal 253 ^a 255 ^aI.D.....		
	penal 253 ^a 260 ^aI.....V.....		
	penal 255 ^a 260 ^aD....V.....		
	penal 255 ^a 261 ^aD.....Y		
mutated sequences containing 2 substitutions	penal 260 ^a 261 ^aVY		
	penal 250 ^a 252 ^a 253 ^a	.E.TI.....		
	penal 250 ^a 252 ^a 255 ^a	.E.T..D.....		
	penal 250 ^a 252 ^a 260 ^a	.E.T.....V.....		
	penal 250 ^a 252 ^a 261 ^a	.E.T.....Y		
	penal 250 ^a 252 ^a 253 ^a	.E.SI.....		
	penal 250 ^a 252 ^a 255 ^a	.E.S..D.....		
	penal 250 ^a 252 ^a 261 ^a	.E.S.....Y		
	penal 250 ^a 253 ^a 255 ^a	.E..I.D.....		
	penal 250 ^a 253 ^a 260 ^a	.E..I.....V.....		
	penal 250 ^a 253 ^a 261 ^a	.E..I.....Y		
	penal 250 ^a 255 ^a 260 ^a	.E....D....V.....		

Table (Se)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5a (continued)			
	mutated amino acid positions that never show any IgE reactivity	sequence	sequence name
Pen a 1 sequence	penal 249-261	LQKEVDRLDELV	penal 249-261
	penal 250 ^a 255 ^a 261 ^a	.E....D....Y	
	penal 250 ^a 260 ^a 261 ^a	.E.....VY	
	penal 252 ^a 253 ^a 255 ^a	...TI.D.....	
	penal 252 ^a 253 ^a 260 ^a	...TI.....V.	
	penal 252 ^a 255 ^a 260 ^a	...T...D....V.	
	penal 252 ^a 255 ^a 261 ^a	...T...D....Y	
	penal 252 ^a 260 ^a 261 ^a	...T.....VY	
	penal 252 ^a 253 ^a 255 ^a	...SI.D.....	
	penal 252 ^a 253 ^a 261 ^a	...SI.....Y	
	penal 252 ^a 255 ^a 261 ^a	...S...D....Y	
	penal 253 ^a 255 ^a 260 ^aI.D....V.	
	penal 253 ^a 255 ^a 261 ^aI.D....Y	
	penal 253 ^a 260 ^a 261 ^aI.....VY	
	penal 255 ^a 260 ^a 261 ^aD....VY	
	penal 250 ^a 252 ^a 253 ^a 255 ^a	.E.TI.D.....	
mutated sequences containing 3 substitutions	penal 250 ^a 252 ^a 253 ^a 260 ^a	.E.TI.....V.	
	penal 250 ^a 252 ^a 253 ^a 261 ^a	.E.TI.....Y	
	penal 250 ^a 252 ^a 255 ^a 260 ^a	.E.T...D....V.	
	penal 250 ^a 252 ^a 255 ^a 261 ^a	.E.T...D....Y	
	penal 250 ^a 252 ^a 260 ^a 261 ^a	.E.T.....VY	
	penal 250 ^a 252 ^a 253 ^a 255 ^a	.E.SI.D.....	
	penal 250 ^a 252 ^a 253 ^a 261 ^a	.E.SI.....Y	
	penal 250 ^a 252 ^a 255 ^a 261 ^a	.E.S...D....Y	
	penal 250 ^a 253 ^a 255 ^a 260 ^a	.E...I.D....V.	
	penal 250 ^a 253 ^a 260 ^a 261 ^a	.E...I.....VY	

Table (5c)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5a (continued)				
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	penal 249-261	LQKVDNLEDELV	penal 249-261	LQKVDNLEDELV
	penal 250 ^s 255 ^s 260 ^v 261 ^v	.E....D....VY		
	penal 252 ^v 253 ^v 255 ^s 260 ^v	...T1.D....V.		
	penal 252 ^v 253 ^v 255 ^s 261 ^v	...T1.D....Y		
	penal 252 ^v 253 ^v 260 ^v 261 ^v	...T1.....VY		
	penal 252 ^v 255 ^s 260 ^v 261 ^v	...T..D....VY		
	penal 253 ^v 255 ^s 260 ^v 261 ^vI.D....VY		
	penal 250 ^s 252 ^v 253 ^v 255 ^s 260 ^v	.E.T1.D....V.		
	penal 250 ^s 252 ^v 253 ^v 255 ^s 261 ^v	.E.T1.D....Y		
	penal 250 ^s 252 ^v 255 ^s 260 ^v 261 ^v	.E.T1.....VY		
mutated sequences containing 4 substitutions	penal 250 ^s 252 ^v 255 ^s 260 ^v 261 ^v	.E.T..D....VY		
	penal 250 ^s 253 ^v 255 ^s 260 ^v 261 ^v	.E..I.D....VY		
	penal 252 ^v 253 ^v 255 ^s 260 ^v 261 ^v	...T1.D....VY		
	penal 252 ^v 253 ^v 255 ^s 260 ^v 261 ^v	...T1.D....VY		
mutated sequences containing 5 substitutions				

Table 5(e)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5a			
	mutated amino acid positions that never show any IgE reactivity	mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence
Pen a 1 sequences	Pen a 1 249-261	LQKVDRLLELV	LQKVDRLLELV
mutated sequences containing 1 substitution	Pen a 1 255 ^aD.....	.E.....
	Pen a 1 260 ^aV.	...T.....
	Pen a 1 261 ^aY	...S.....
		I.....
mutated sequences containing 2 substitutions	Pen a 1 250 ^a 252 ^a	.E.S.....	.E.T.....
	Pen a 1 250 ^a 255 ^a	.E.....D.....	.E..I.....
	Pen a 1 250 ^a 260 ^a	.E.....V.	.E....D.....
	Pen a 1 252 ^a 255 ^a	...T..D.....	...TI.....
	Pen a 1 252 ^a 260 ^a	...T.....V.	...SI.....
	Pen a 1 252 ^a 255 ^a	...S..D.....I.....Y
	Pen a 1 252 ^a 261 ^a	...S.....Y	
	Pen a 1 253 ^a 255 ^aI.D.....	
	Pen a 1 253 ^a 260 ^aI.....V.	
	Pen a 1 255 ^a 260 ^aD.....V.	
	Pen a 1 255 ^a 261 ^aD.....Y	
	Pen a 1 260 ^a 261 ^aVY	
mutated sequences containing 3 substitutions	Pen a 1 250 ^a 252 ^a 253 ^a	.E.TI.....	
	Pen a 1 250 ^a 252 ^a 255 ^a	.E..D.....	
	Pen a 1 250 ^a 252 ^a 260 ^a	.E.T.....V.	
	Pen a 1 250 ^a 252 ^a 261 ^a	.E.T.....Y	
	Pen a 1 250 ^a 252 ^a 253 ^a	.E.SI.....	
	Pen a 1 250 ^a 252 ^a 255 ^a	.E.S..D.....	
	Pen a 1 250 ^a 252 ^a 261 ^a	.E.S.....Y	
	Pen a 1 250 ^a 253 ^a 255 ^a	.E..I.D.....	
	Pen a 1 250 ^a 253 ^a 260 ^a	.E..I.....V.	
	Pen a 1 250 ^a 253 ^a 261 ^a	.E..I.....Y	
	Pen a 1 250 ^a 255 ^a 260 ^a	.E.....Y	
	Pen a 1 250 ^a 255 ^a 260 ^a	.E.....V.	

Table 5(e)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5a (continued)			
	mutated amino acid positions that never show any IgE reactivity	sequence	sequence
	sequence name	sequence	sequence
Pen a 1 sequence	Pen a 1 249-261	LQKVDRLDELV	LQKVDRLDELV
	Pen a 1 250 ^a 255 ^a 261 ^v	.E....D....Y	
mutated sequences containing 3 substitutions	Pen a 1 250 ^a 260 ^v 261 ^v	.E.....VY	
	Pen a 1 252 ^a 253 ^v 255 ^a	...TI.D.....	
	Pen a 1 252 ^a 253 ^v 260 ^v	...TI.....V.	
	Pen a 1 252 ^a 255 ^a 260 ^v	...Y..D....V.	
	Pen a 1 252 ^a 255 ^a 261 ^v	...T..D....Y	
	Pen a 1 252 ^a 260 ^v 261 ^v	...T.....VY	
	Pen a 1 252 ^a 253 ^v 255 ^a	...SI.D.....	
	Pen a 1 252 ^a 253 ^v 261 ^v	...SI.....Y	
	Pen a 1 252 ^a 255 ^a 261 ^v	...S..D....Y	
	Pen a 1 253 ^v 255 ^a 260 ^vI.D....V.	
	Pen a 1 253 ^v 255 ^a 261 ^vI.D....Y	
	Pen a 1 253 ^v 260 ^v 261 ^vI.....VY	
	Pen a 1 255 ^a 260 ^v 261 ^vD....VY	
	Pen a 1 250 ^a 252 ^a 253 ^v 255 ^a	.E.TI.D.....	
	Pen a 1 250 ^a 252 ^a 253 ^v 260 ^v	.E.TI.....V.	
	Pen a 1 250 ^a 252 ^a 253 ^v 261 ^v	.E.TI.....Y	
mutated sequences containing 4 substitutions	Pen a 1 250 ^a 252 ^a 255 ^a 260 ^v	.E.T..D....V.	
	Pen a 1 250 ^a 252 ^a 255 ^a 261 ^v	.E.T..D....Y	
	Pen a 1 250 ^a 252 ^a 260 ^v 261 ^v	.E.T.....VY	
	Pen a 1 250 ^a 252 ^a 253 ^v 255 ^a	.E.SI.D.....	
	Pen a 1 250 ^a 252 ^a 253 ^v 261 ^v	.E.SI.....Y	
	Pen a 1 250 ^a 252 ^a 255 ^a 261 ^v	.E.S..D....Y	
	Pen a 1 250 ^a 253 ^v 255 ^a 260 ^v	.E..I.D....V.	
	Pen a 1 250 ^a 253 ^v 260 ^v 261 ^v	.E..I.....VY	

Table 5(e)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5a (continued)				
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	Pen a 1 249-261	LQKEVDRLDELV	Pen a 1 249-261	LQKEVDRLDELV
	Pen a 1 250 ^a 255 ^a 260 ^a 261 ^a	.E....D....VY		
	Pen a 1 252 ^a 253 ^a 255 ^a 260 ^a	...TI..D....V.		
	Pen a 1 252 ^a 253 ^a 255 ^a 261 ^a	...TI..D....Y		
	Pen a 1 252 ^a 253 ^a 260 ^a 261 ^a	...TI.....VY		
	Pen a 1 252 ^a 255 ^a 260 ^a 261 ^a	...T...D....VY		
mutated sequences containing 4 substitutions	Pen a 1 253 ^a 255 ^a 260 ^a 261 ^aI..D....VY		
	Pen a 1 250 ^a 252 ^a 253 ^a 255 ^a 260 ^a	.E..TI..D....V.		
	Pen a 1 250 ^a 252 ^a 253 ^a 255 ^a 261 ^a	.E..TI..D....Y		
	Pen a 1 250 ^a 252 ^a 253 ^a 260 ^a 261 ^a	.E..TI.....VY		
	Pen a 1 250 ^a 252 ^a 255 ^a 260 ^a 261 ^a	.E..T...D....VY		
	Pen a 1 250 ^a 253 ^a 255 ^a 260 ^a 261 ^a	.E...I..D....VY		
mutated sequences containing 5 substitutions	Pen a 1 252 ^a 253 ^a 255 ^a 260 ^a 261 ^a	...TI..D....VY		

Table 5e)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5a				
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
unmodified Pen a 1 sequence	Pen a 1 273-281	ELDQTFSEL	Pen a 1 273-281	ELDQTFSEL
	Pen a 1 277 ^aA....	Pen a 1 276 ^a	...M....
	Pen a 1 278 ^bL...	Pen a 1 275 ^a 281	..M.....
	Pen a 1 280 ^cD.		
	Pen a 1 281 ^dN		
mutated sequences containing 2 substitutions	Pen a 1 281 ^eI		
	Pen a 1 276 ^a 277 ^a	...HA....	Pen a 1 276 ^a 279 ^a	...M...T..
	Pen a 1 276 ^a 278 ^b	...H.L...	Pen a 1 276 ^a 279 ^a	...H...N..
	Pen a 1 276 ^a 280 ^c	...H...D.	Pen a 1 276 ^a 279 ^a	...H...N..
	Pen a 1 276 ^a 281 ^d	...H...N		
	Pen a 1 277 ^a 278 ^bAL...		
	Pen a 1 277 ^a 279 ^aA.N..		
	Pen a 1 277 ^a 280 ^cA...D.		
	Pen a 1 277 ^a 281 ^dA...M		
	Pen a 1 278 ^b 279 ^aLN..		

Table 5(f)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5b (continued)			
	mutated amino acid positions that never show any IgE reactivity	sequence	mutated amino acid positions that show reduced and/or abolished IgE reactivity
Pen a 1 sequence	sequence name	sequence	sequence name
mutated sequences containing 1 substitution	Pen a 1 266-273	KYKSTIDE	Pen a 1 266-273
	Pen a 1 269 ¹	...E....	
	Pen a 1 269 ²	...D....	
	Pen a 1 269 ³	...C....	
	Pen a 1 269 ⁴	...A....	
	Pen a 1 270 ¹	...W...	
	Pen a 1 270 ²	...T...	
	Pen a 1 270 ³	...S...	
	Pen a 1 270 ⁴	...R...	
	Pen a 1 270 ⁵	...Q...	
	Pen a 1 270 ⁶	...P...	
	Pen a 1 270 ⁷	...N...	
	Pen a 1 270 ⁸	...M...	
	Pen a 1 270 ⁹	...K...	
	Pen a 1 270 ¹⁰	...H...	
	Pen a 1 270 ¹¹	...G...	
	Pen a 1 270 ¹²	...F...	
	Pen a 1 270 ¹³	...E...	
	Pen a 1 270 ¹⁴	...D...	
	Pen a 1 270 ¹⁵	...C...	
	Pen a 1 270 ¹⁶	...A...	
	Pen a 1 271 ¹	...P...	
	Pen a 1 272 ¹	...R...	
	Pen a 1 272 ²	...P...	
	Pen a 1 272 ³	...K...	

Table (58)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5b (continued)				
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
Pen a 1 sequence	Pen a 1 266-273	KYKSTDE	Pen a 1 266-273	KYKSTDE
	Pen a 1 269 ^r 271 ^c	...F.C...	Pen a 1 269 ^a 271 ^a	...A.S...
	Pen a 1 269 ^r 273 ^a	...F...D	Pen a 1 269 ^a 272 ^a	...A...E.
			Pen a 1 271 ^a 272 ^aSE.
mutated sequences containing 2 substitutions			Pen a 1 271 ^c 273 ^aC.D
	Pen a 1 269 ^r 271 ^c 273 ^a	...F.C.D	Pen a 1 269 ^a 271 ^a 272 ^a	...A.SE.
mutated sequences containing 3 substitutions				

Table 5(f)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5b				
Pen a 1 sequence	mutated amino acid positions that never show any IgG reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
mutated sequences containing 1 substitution	Pen a 1 266-273	KYKSTIDE	Pen a 1 266-273	KYKSTIDE
	Pen a 1 269 ^a	...E....	Pen a 1 269 ^a	...A....
	Pen a 1 266 ^a		Pen a 1 271 ^aS...
	Pen a 1 266 ^a	Y.....	Pen a 1 266 ^a	N.....
	Pen a 1 266 ^a	W.....	Pen a 1 267 ^a	H.....
	Pen a 1 266 ^a	V.....	Pen a 1 268 ^a	..Y.....
	Pen a 1 266 ^a	T.....	Pen a 1 268 ^a	..M.....
	Pen a 1 266 ^a	B.....	Pen a 1 268 ^a	..T.....
	Pen a 1 266 ^a	R.....	Pen a 1 268 ^a	..S.....
	Pen a 1 266 ^a	Q.....	Pen a 1 268 ^a	..N.....
	Pen a 1 266 ^a	P.....	Pen a 1 268 ^a	..L.....
	Pen a 1 266 ^a	M.....	Pen a 1 268 ^a	..I.....
	Pen a 1 266 ^a	L.....	Pen a 1 268 ^a	..H.....
	Pen a 1 266 ^a	I.....	Pen a 1 268 ^a	..G.....
	Pen a 1 266 ^a	H.....	Pen a 1 268 ^a	..F.....
	Pen a 1 266 ^a	G.....	Pen a 1 268 ^a	..A.....
	Pen a 1 266 ^a	F.....	Pen a 1 269 ^a	...T....
	Pen a 1 266 ^a	E.....	Pen a 1 269 ^a	...G....
	Pen a 1 266 ^a	D.....	Pen a 1 270 ^aY....
	Pen a 1 266 ^a	C.....	Pen a 1 270 ^aV....
	Pen a 1 266 ^a	A.....	Pen a 1 270 ^aL....
	Pen a 1 267 ^a	V.....	Pen a 1 270 ^aF....
	Pen a 1 267 ^a	T.....	Pen a 1 270 ^aE....
	Pen a 1 267 ^a	S.....	Pen a 1 271 ^aM....
	Pen a 1 267 ^a	R.....	Pen a 1 271 ^aK....
	Pen a 1 267 ^a	Q.....	Pen a 1 272 ^aY....
	Pen a 1 267 ^a	P.....	Pen a 1 272 ^aM....
	Pen a 1 267 ^a	M.....	Pen a 1 272 ^aV....

Table 5(f)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5b (continued)			
Pen a 1 sequence	mutated amino acid positions that never show any IgE reactivity		sequence
	sequence name	sequence	
mutated sequences containing 1 substitution	Pen a 1 266-273	KYKSTDE	Pen a 1 266-273
	Pen a 1 267 ^a	.L.....	Pen a 1 272 ^a
	Pen a 1 267 ^a	.K.....	Pen a 1 272 ^a
	Pen a 1 267 ^a	.I.....	Pen a 1 272 ^a
	Pen a 1 267 ^a	.G.....	Pen a 1 272 ^a
	Pen a 1 267 ^a	.E.....	Pen a 1 272 ^a
	Pen a 1 267 ^a	.D.....	Pen a 1 272 ^a
	Pen a 1 267 ^a	.C.....	Pen a 1 272 ^a
	Pen a 1 267 ^a	.A.....	Pen a 1 272 ^a
	Pen a 1 268 ^a	.V.....	Pen a 1 273 ^a
	Pen a 1 268 ^a	.P.....	Pen a 1 273 ^a
	Pen a 1 268 ^a	.E.....	Pen a 1 273 ^a
	Pen a 1 268 ^a	.D.....	Pen a 1 273 ^a
	Pen a 1 268 ^a	.C.....	Pen a 1 273 ^a
	Pen a 1 269 ^a	...Y....	Pen a 1 273 ^a
	Pen a 1 269 ^a	...W....	Pen a 1 273 ^a
	Pen a 1 269 ^a	...V....	Pen a 1 273 ^a
	Pen a 1 269 ^a	...T....	Pen a 1 273 ^a
	Pen a 1 269 ^a	...R....	Pen a 1 273 ^a
	Pen a 1 269 ^a	...Q....	Pen a 1 273 ^a
	Pen a 1 269 ^a	...P....	Pen a 1 273 ^a
	Pen a 1 269 ^a	...M....	Pen a 1 273 ^a
	Pen a 1 269 ^a	...L....	Pen a 1 273 ^a
	Pen a 1 269 ^a	...K....	Pen a 1 273 ^a
	Pen a 1 269 ^a	...I....	Pen a 1 273 ^a
	Pen a 1 269 ^a	...H....	Pen a 1 273 ^a
	Pen a 1 269 ^a	...G....	Pen a 1 273 ^a
	Pen a 1 269 ^a	...F....	Pen a 1 273 ^a
			KYKSTDE
		T.
		M.
		L.
		I.
		H.
		F.
		C.
		A.
		Y.
		W.
		V.
		T.
		S.
		R.
		Q.
		P.
		N.
		M.
		L.
		I.
		H.
		G.
		F.
		A.

Table 5 (g)

Mutated Pen a 1 Positions that Reduce or Abolish IgE Antibody Reactivity to Epitope 5c (continued)				
	mutated amino acid positions that never show any IgE reactivity		mutated amino acid positions that show reduced and/or abolished IgE reactivity	
	sequence name	sequence	sequence name	sequence
unmodified Pen a 1 sequence	Pen a 1 273-281	ELDQTFSEL	Pen a 1 273-281	ELDQTFSEL
	Pen a 1 280 ^a 281 ^aDM		
	Pen a 1 276 ^a 277 ^a	...NA....		
	Pen a 1 276 ^a 278 ^a	...N..L....		
	Pen a 1 276 ^a 280 ^a	...N...D.		
	Pen a 1 276 ^a 281 ^a	...N....I		
	Pen a 1 277 ^a 281 ^aA...I		
	Pen a 1 278 ^a 281 ^aI...I		
	Pen a 1 279 ^a 281 ^aN..I		
	Pen a 1 280 ^a 281 ^aDI		
mutated sequences containing 3 substitutions	Pen a 1 276 ^a 281 ^a	...N....M		
			Pen a 1 273 ^a 276 ^a 279 ^a	D..M..T..

location	recombinant peptide library	overlapping, synthetic peptides
N-terminus		Pen a 1 1-16 NDAIKKKKQAMKLEK Pen a 1 19-33 SNAEGGVAAALNRRIQ
center	Pen a 1 136-148 SQEERMDALENQL Pen a 1 157-169 EADPKYGEVAPKL Pen a 1 167-179 RRLAMVEADLERA	Pen a 1 109-123 ATTALAERSQAQDES Pen a 1 121-135 QESERMRKYLENRSQ Pen a 1 133-147 RSLSDQERMDALENG Pen a 1 137-201 ESKIVELDEELAVVG
C-terminus	Pen a 1 262-292 NENENYKSITQELDQTFSELS	Pen a 1 217-231 REEAYKEQIKTITNK Pen a 1 241-255 FAERSVQKLCQKEVDR Pen a 1 261-267 VDRLEDELVNSKENEY

6
 Table X: IgE-reactive peptides of Pen a 1 identified with a recombinant peptide library and synthetic, overlapping peptides

Table 67

This table shows all the substitutions (position, pos; substituting amino acid, aa) that can be considered to reduce or abolish the IgE antibody reactivity of major and minor IgE-binding regions. In addition to the five major region, minor IgE-reactive regions, defined as regions to which at least one allergic subject shows strong IgE antibody reactivity.

minor region 1		major region 1		minor region 2		major region 2		minor region 3		major region 3		minor region 4		major region 4		minor region 5		major region 5		minor region 6		major region 6	
pos	aa	pos	aa	pos	aa	pos	aa	pos	aa	pos	aa	pos	aa	pos	aa	pos	aa	pos	aa	pos	aa	pos	aa
250	E	43	L	70	V	95	V	103	Q	125	Q	125	Q	135	K	146	I	188	G	220	K	250	E
252	S	44	I	71	V	95	F	107	A	127	M	127	M	140	K	153	K	190	C	222	E	252	S
255	D	45	Q	74	S	98	E	110	L	130	I	130	I	142	E	154	H	192	D	224	E	255	D
260	V	46	M			100	D	111	Q	134	A	134	A	144	Q	155	I	198	K	227	V	260	V
267	F	49	K					114	E	135	Q	135	Q			163	E	199	T	230	D	267	F
277	A	50	L					117	E	135	Q	135	Q			170	V	201	T			277	A
278	L	51	K							135	M	135	M			171	I					278	L
280	Q	52	G							135	S	135	S			172	I					280	Q
281	M	53	T													174	S					281	M
281	I	55	D													179	S					281	I
31	A	56	B													185	I						
33	E															186	S						
																188	G						

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/30968

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : Please See Extra Sheet.

US CL : 424/184.1, 536/23.5, 435/69.1, 435/320.1, 800/3, 530/387.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/184.1, 536/23.5, 435/69.1, 435/320.1, 800/3, 530/387.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,449,669 A (METCALF ET AL) 12 September 1995(12/9/95), see entire document.	1-7, 12-26, 28-42
X	REESE, G. Int. Arch. Allergy Immunol. 1997, Vol 113, pages 240-242., See entire document.	8-11, 27, 42
X	REESE, G. J. Allergy Clin. Immunol. 1995, Vol 95, No. 1, Part 2, page 331. See abstract.	44
Y	US 6,118,044A (KARASUYAMA et al) 12 September 2000 (12/9/00), Col. 2, lines 14-39.	43



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

26 DECEMBER 2000

Date of mailing of the international search report

12 MAR 2001

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

PHUONG N. HUYNH

Telephone No. (703) 308-0168

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/30968

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (7):

A61K 39/00, C07H 21/04, C12P 21/06, C12N 15/00, G01N 33/00, C07K 16/00

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN, MEDLINE, BIOSIS, CAPLUS, EMBASE, SCISEARCH

Vaccine, crustacea, tropomyosin, IgE, transgenic shrimp, crustacea tropomyosin antibody, recombinant peptide, allergen, IgE mediated hypersensitivity, Pen a 1

